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(54) **COMPOUNDS FOR TREATING SPINAL MUSCULAR ATROPHY**

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C07D 407/04 (2006.01)

C07D 409/14 (2006.01)

C07D 487/04 (2006.01)

C07D 497/04 (2006.01)

C07D 405/04 (2006.01)

C07D 409/04 (2006.01)

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(52) **U.S. Cl.**

CPC **C07D 513/04** (2013.01); **C07D 311/76** (2013.01); **C07D 405/04** (2013.01); **C07D 405/14** (2013.01); **C07D 407/04** (2013.01); **C07D 409/04** (2013.01); **C07D 409/14** (2013.01); **C07D 471/04** (2013.01); **C07D 487/04** (2013.01); **C07D 497/04** (2013.01)

(58) **Field of Classification Search**

CPC **C07D 311/76**
See application file for complete search history.

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(57) **ABSTRACT**

Provided herein are compounds, compositions thereof and uses therewith for treating spinal muscular atrophy.

11 Claims, 21 Drawing Sheets

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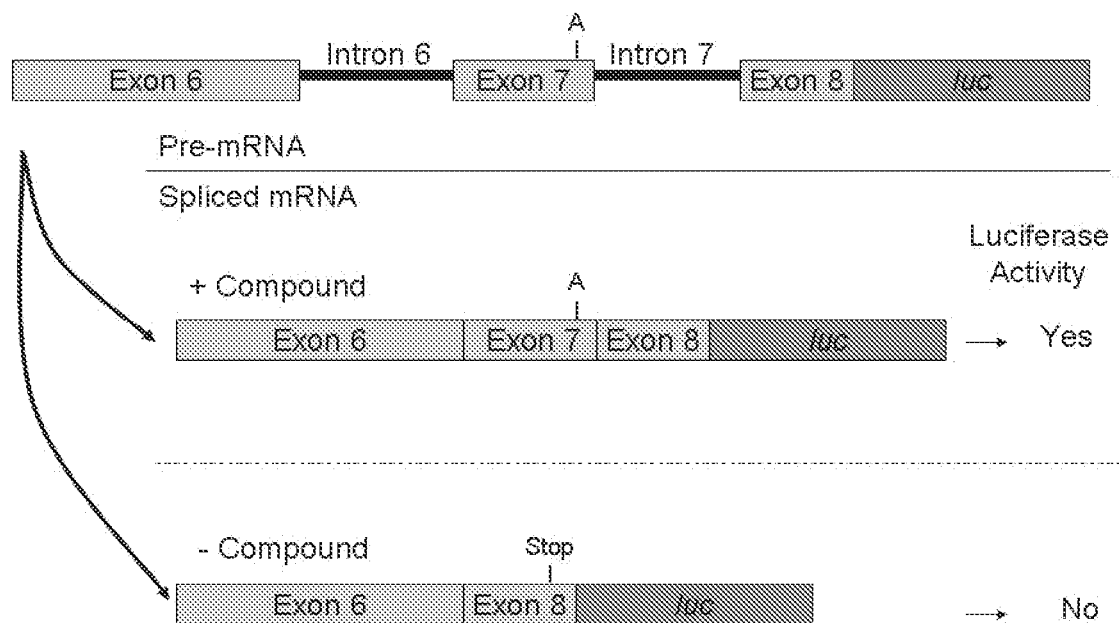


Figure 1

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Figure 2a

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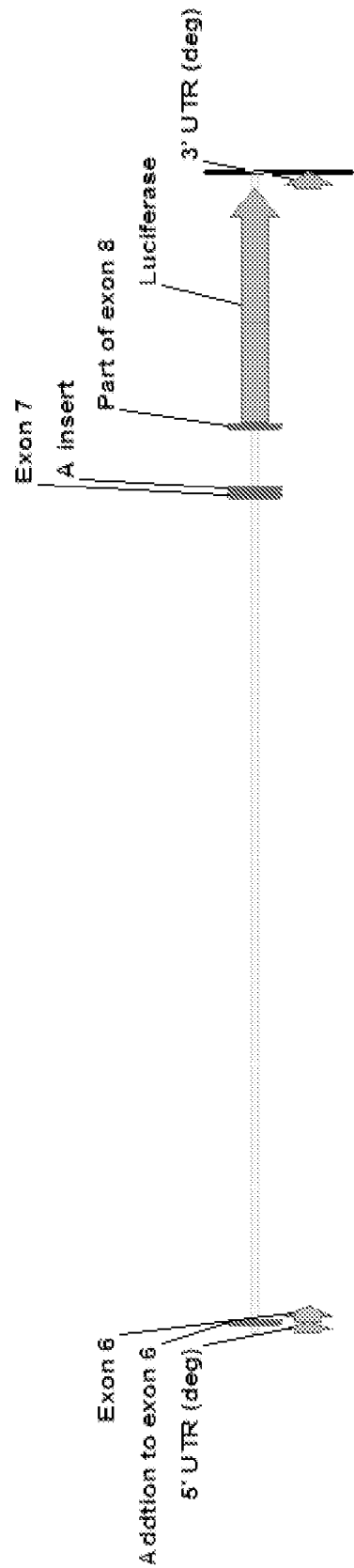


Figure 2b

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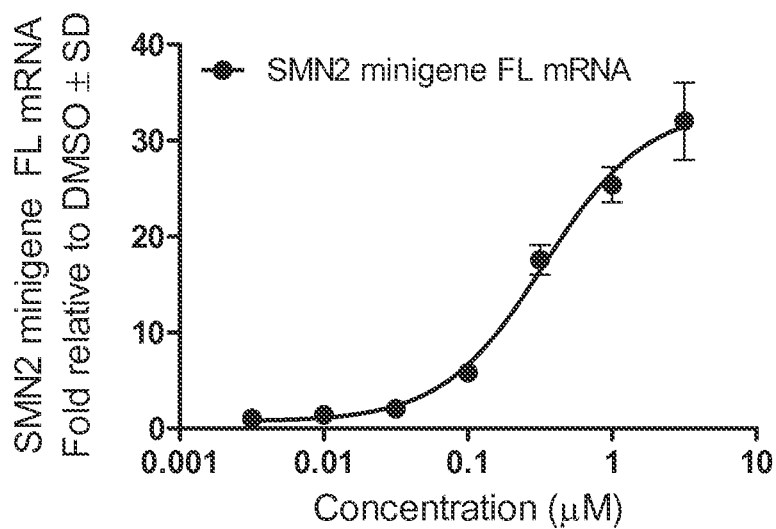


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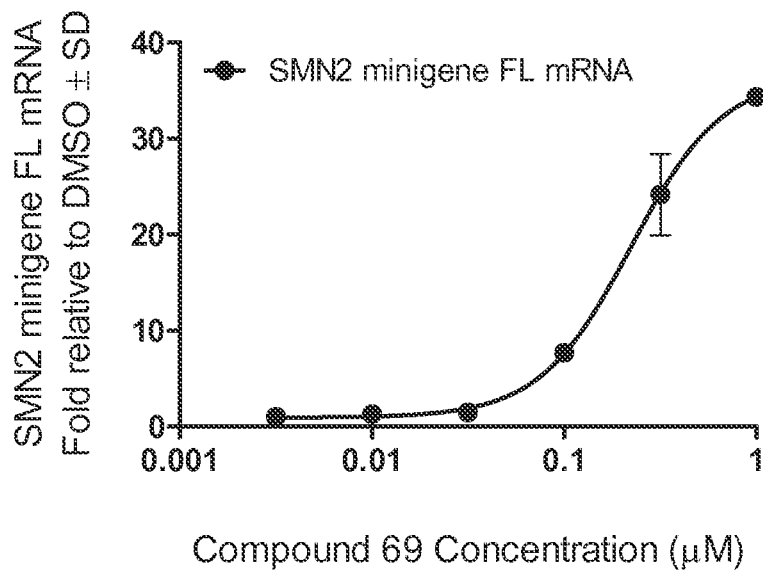


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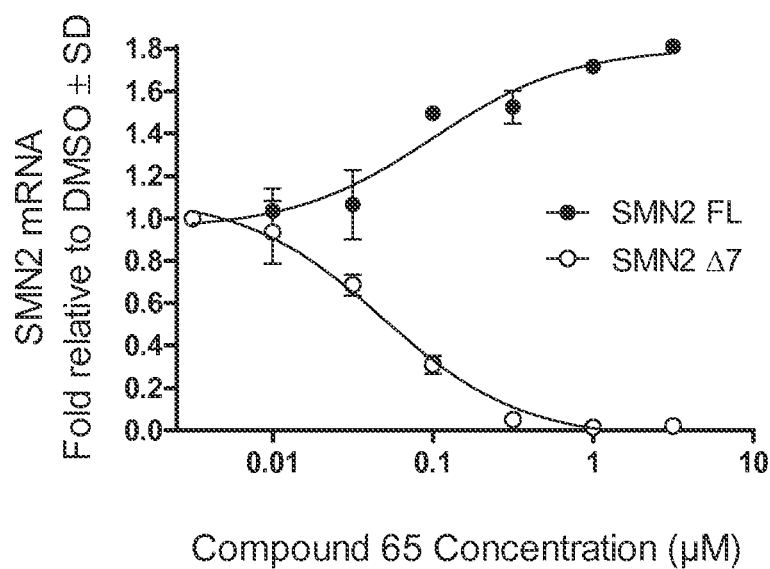


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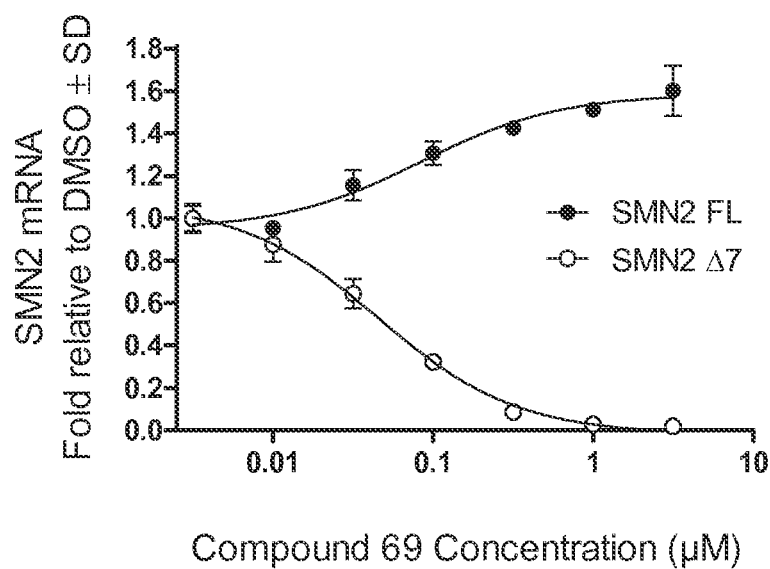


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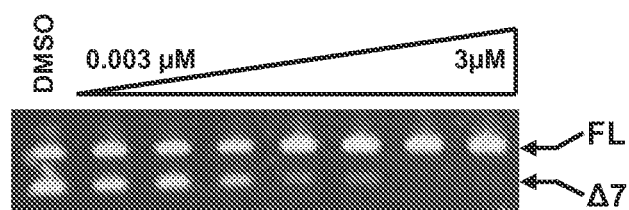


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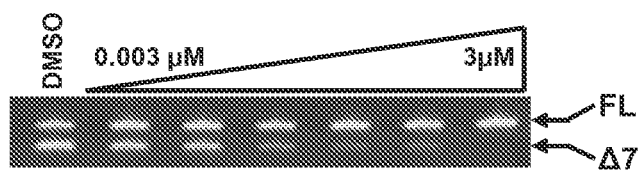


Figure 5b

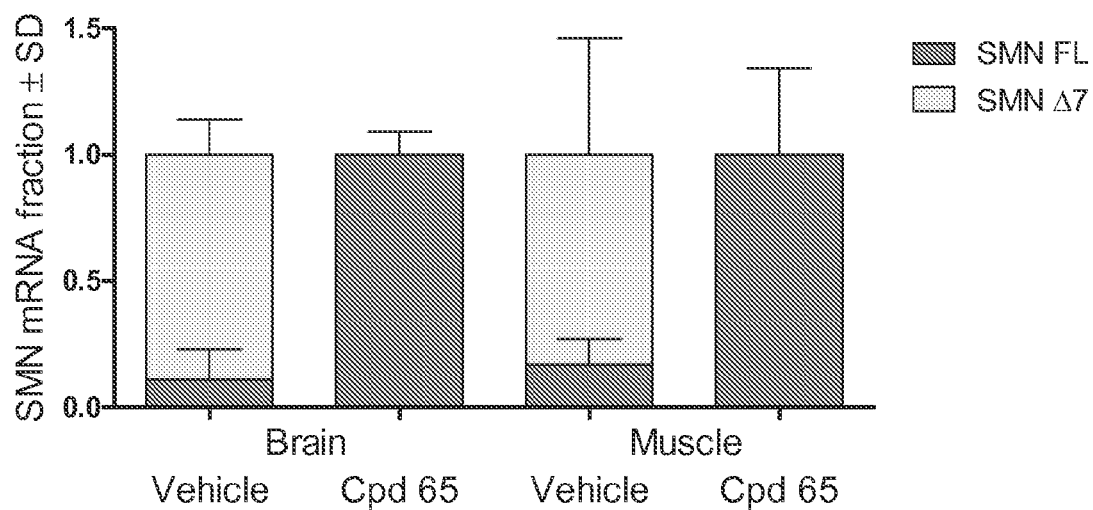


Figure 6a

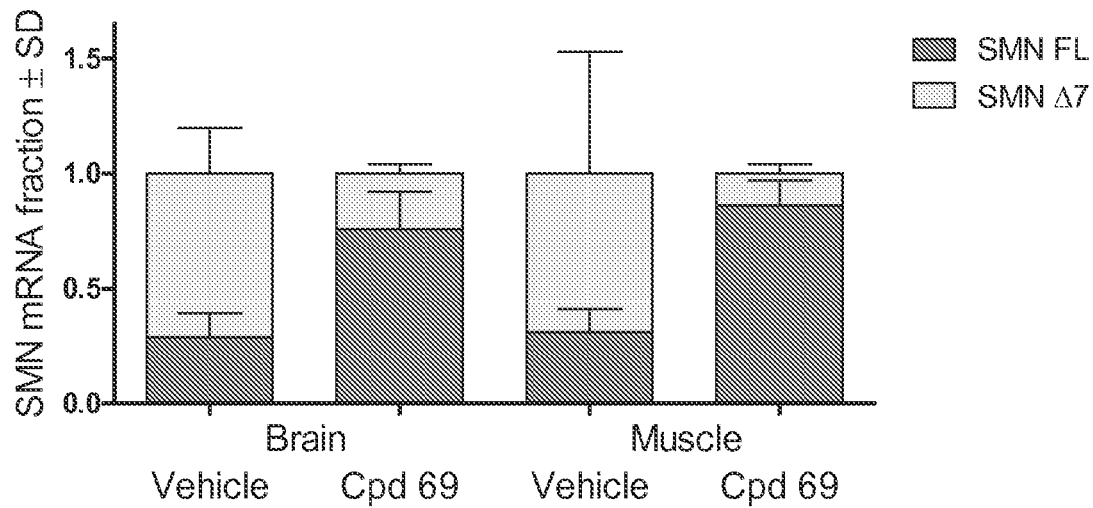


Figure 6b

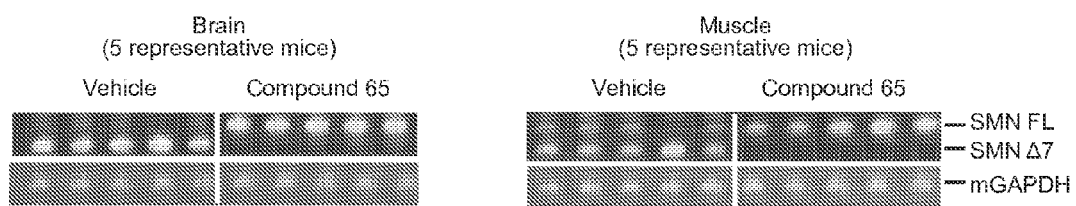


Figure 7

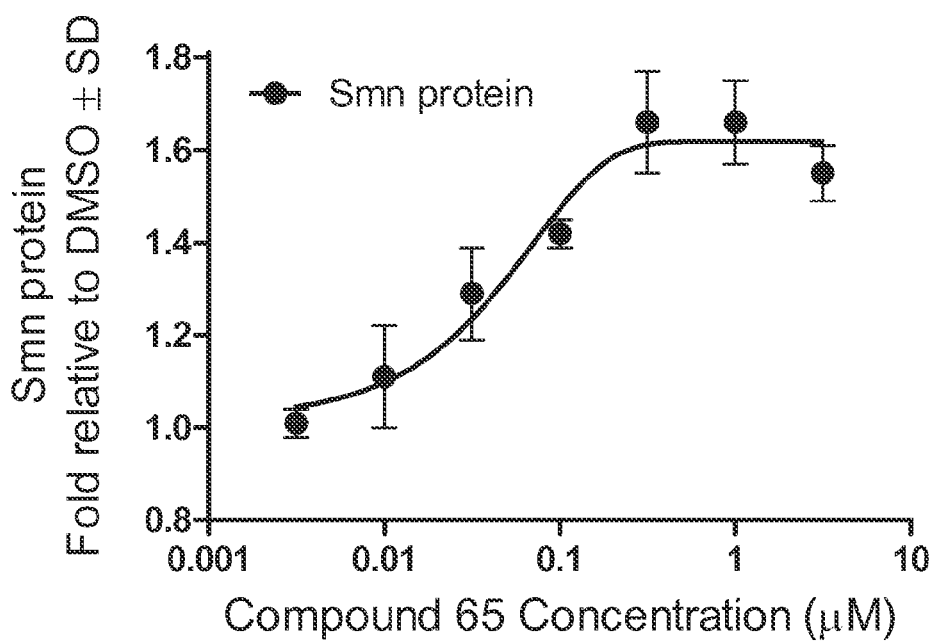


Figure 8a

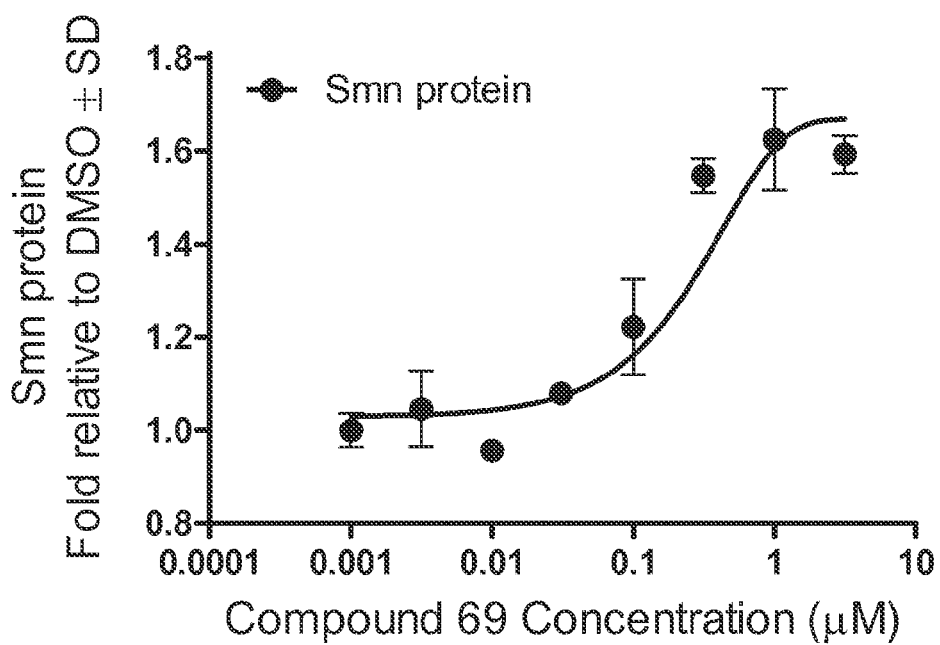


Figure 8b

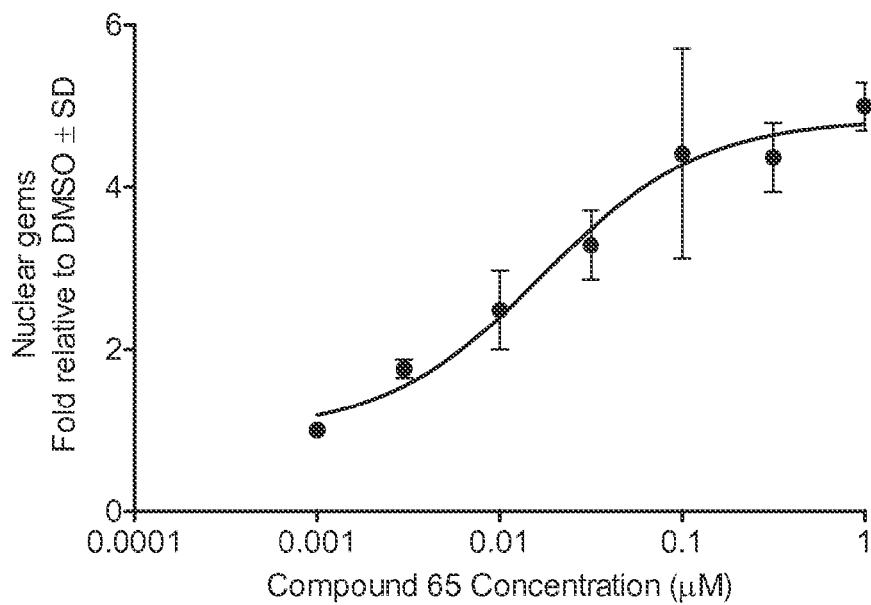


Figure 9a

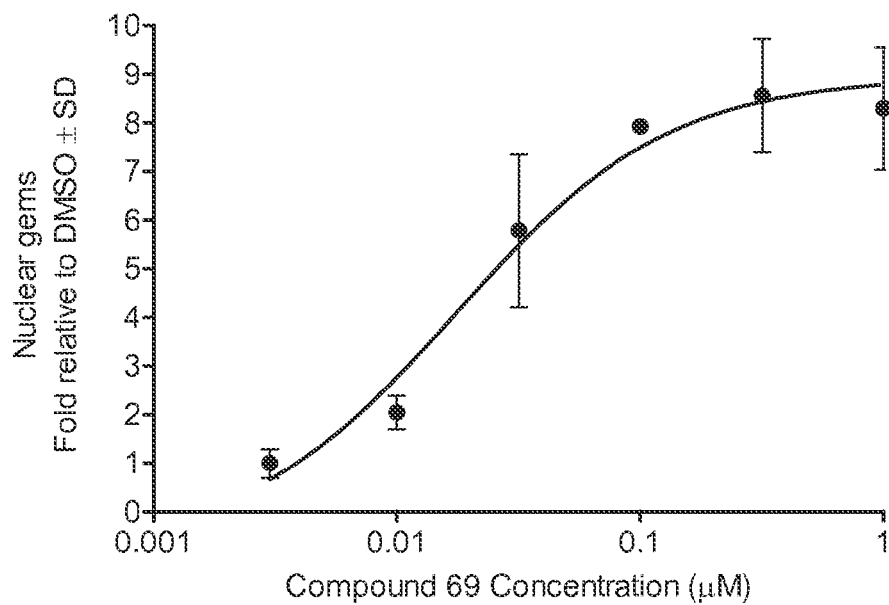


Figure 9b

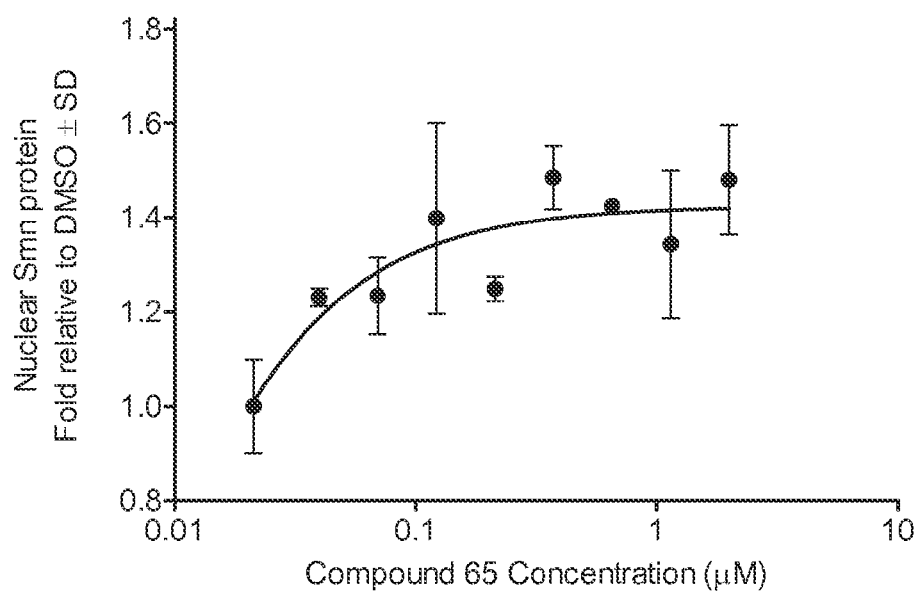


Figure 10a

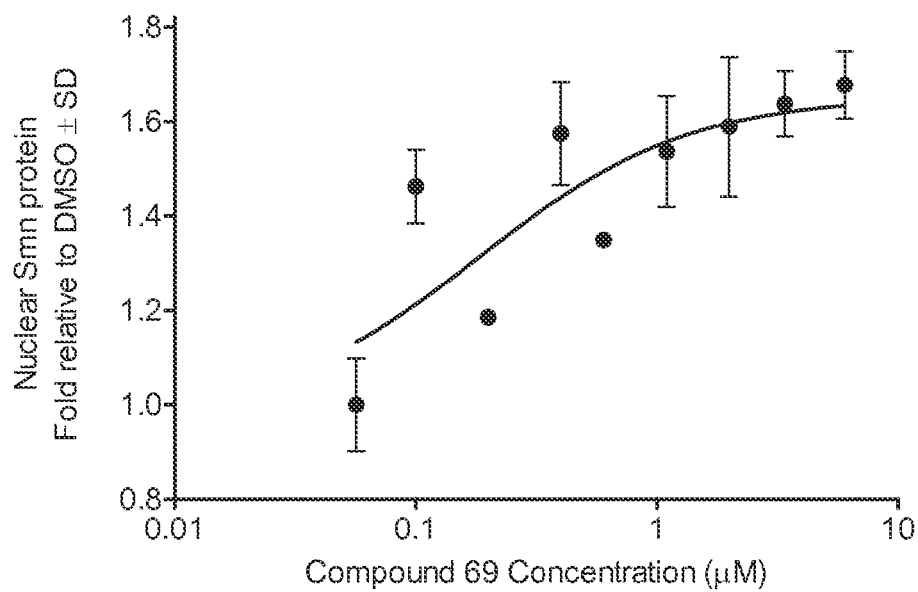


Figure 10b

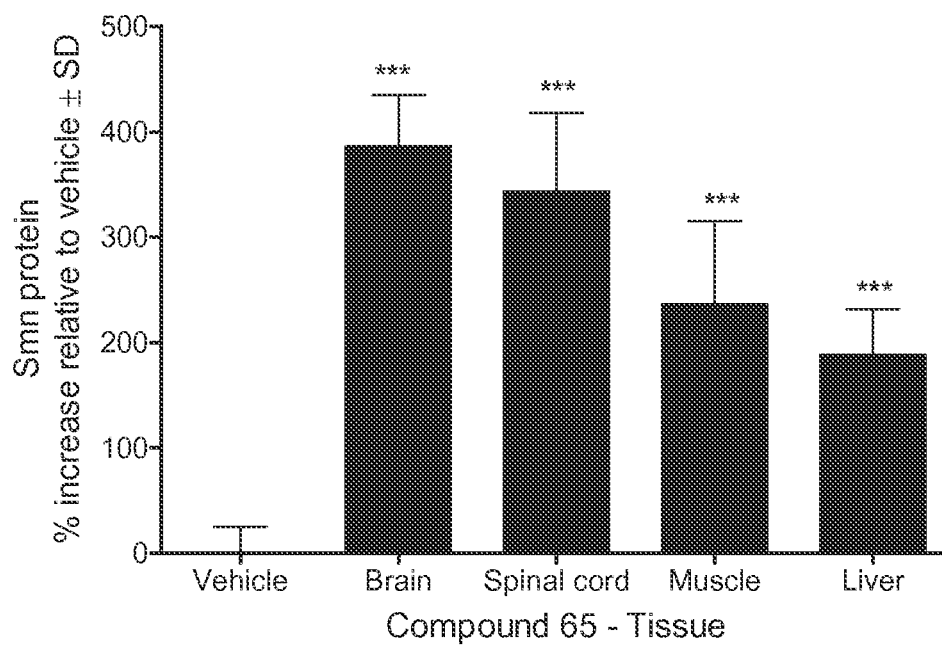


Figure 11a

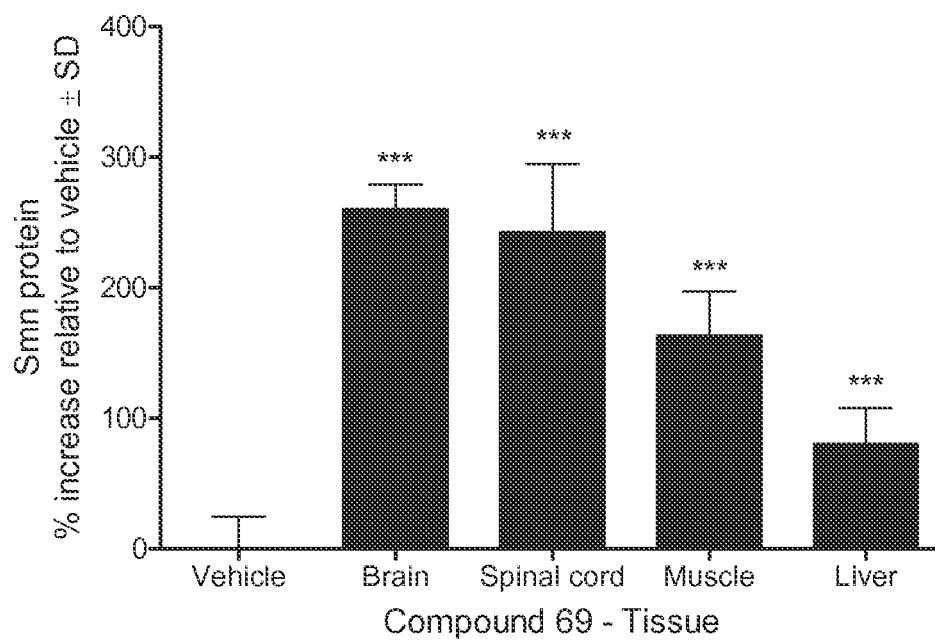


Figure 11b

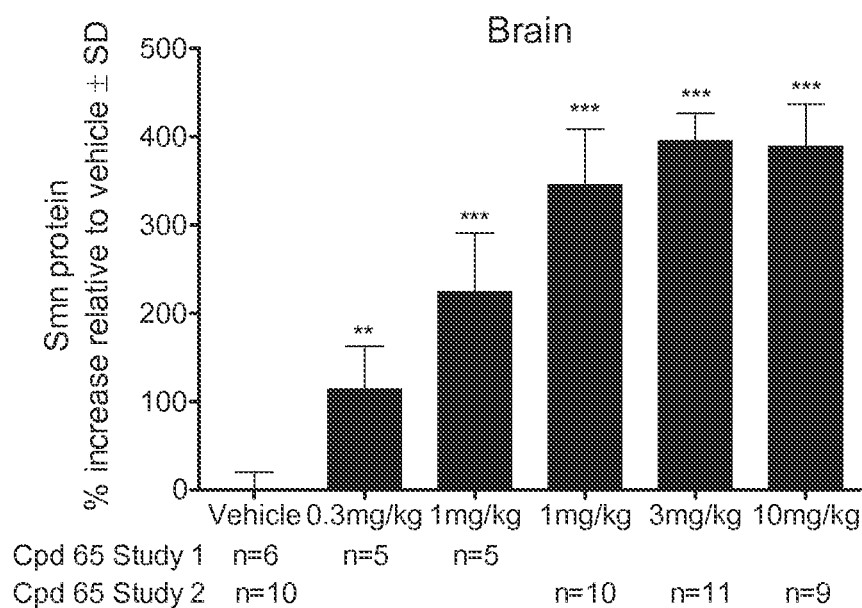


Figure 12a

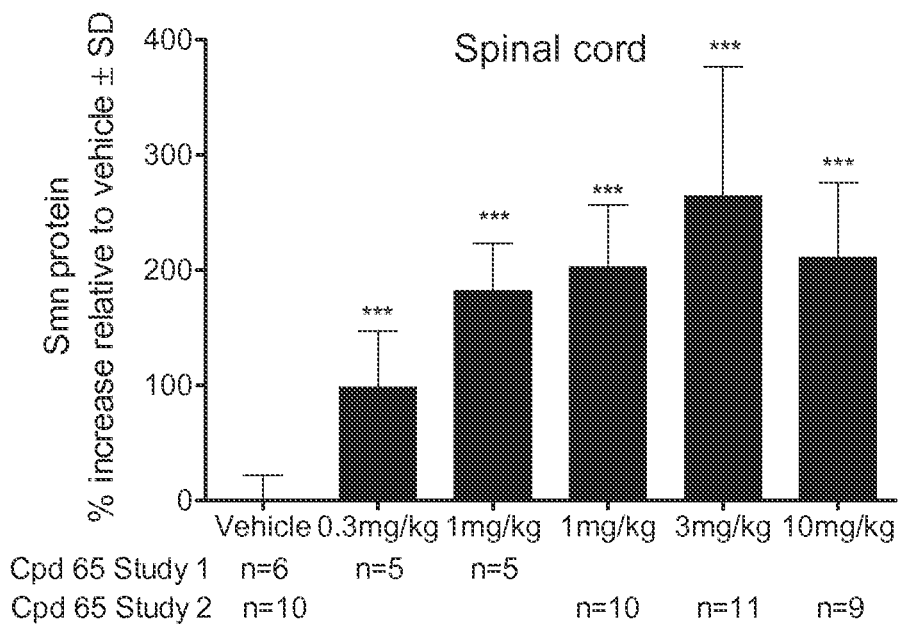


Figure 12b

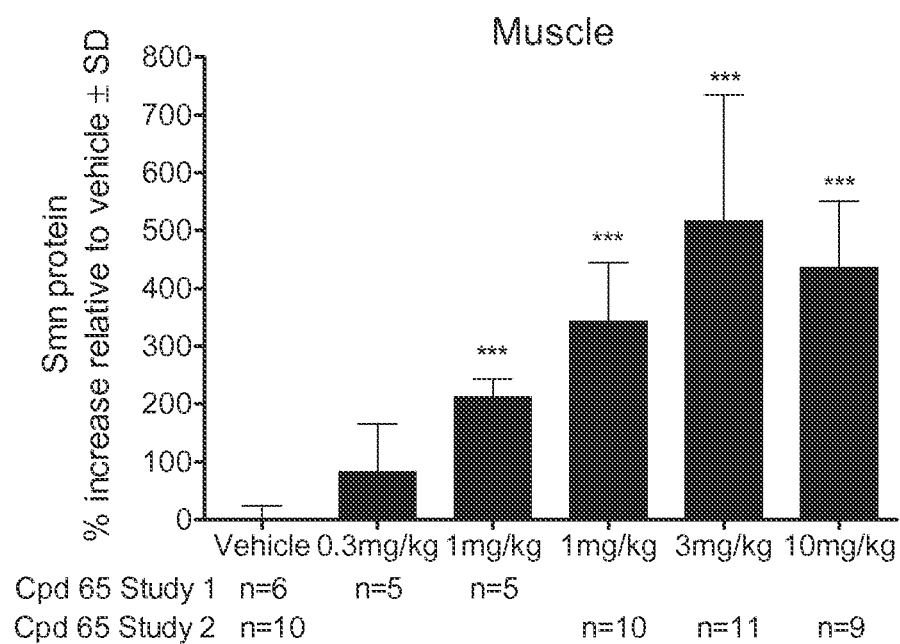


Figure 12c

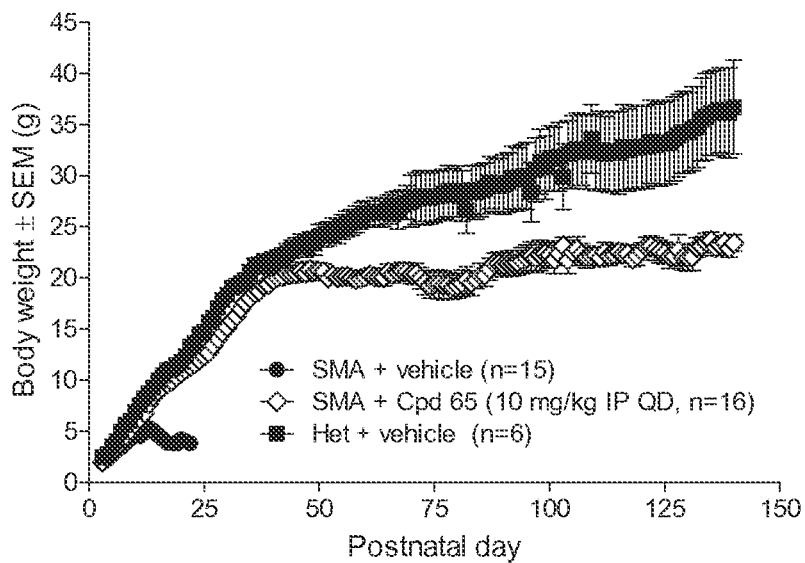


Figure 13a

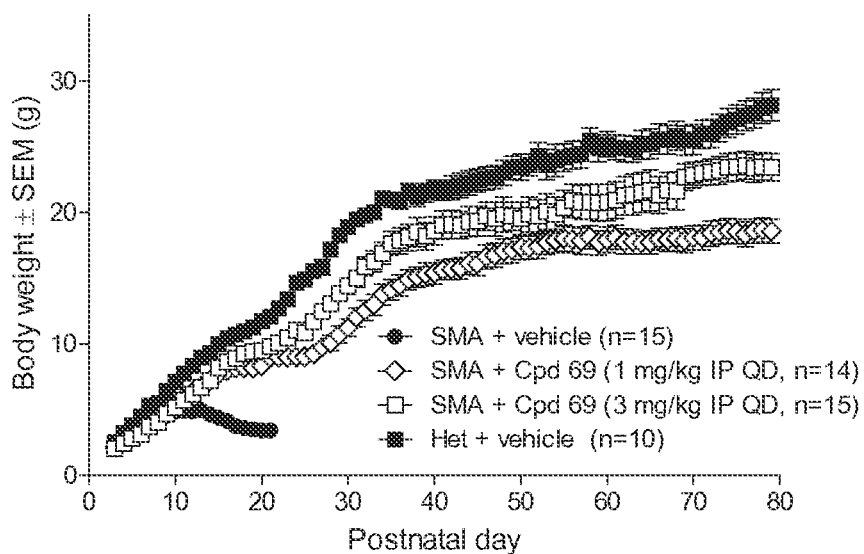


Figure 13b

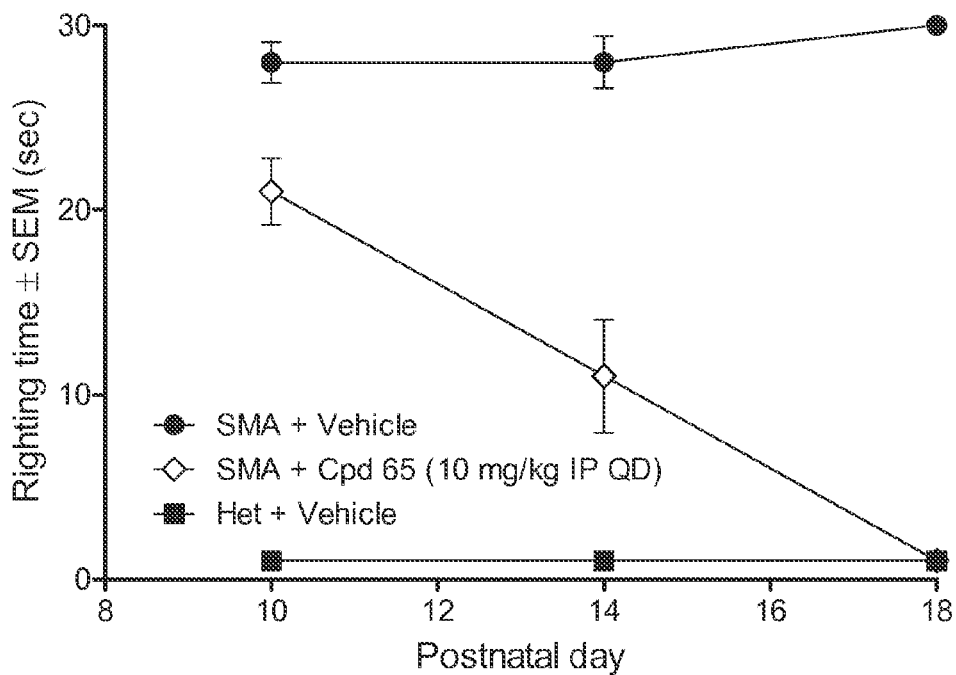


Figure 14a

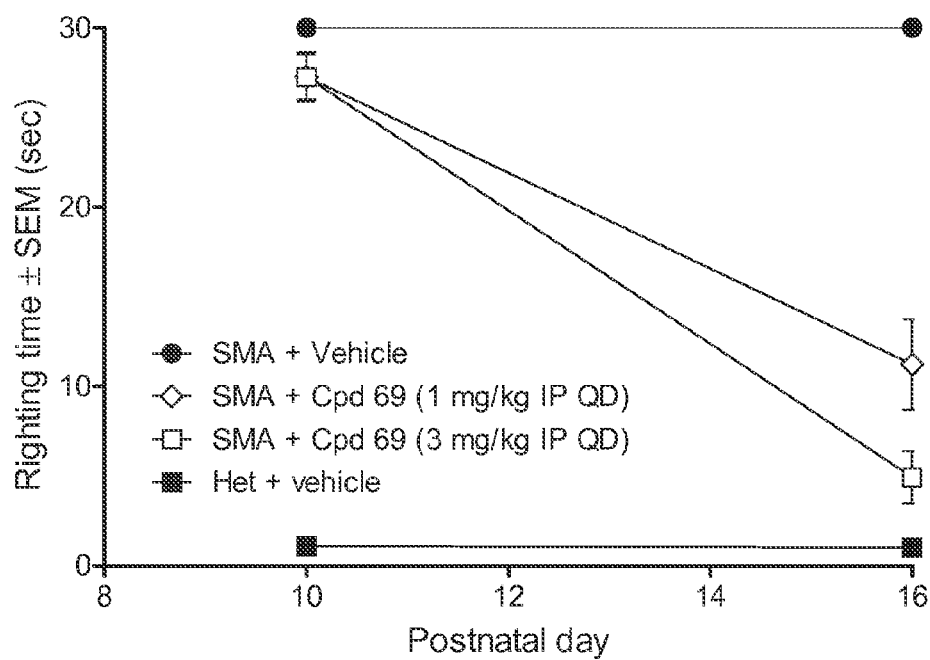


Figure 14b

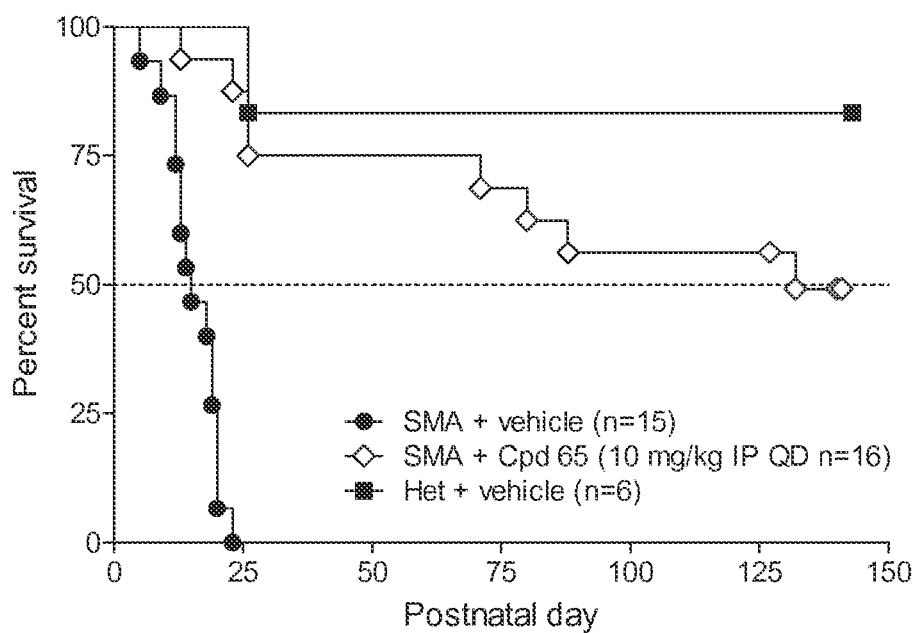


Figure 15a

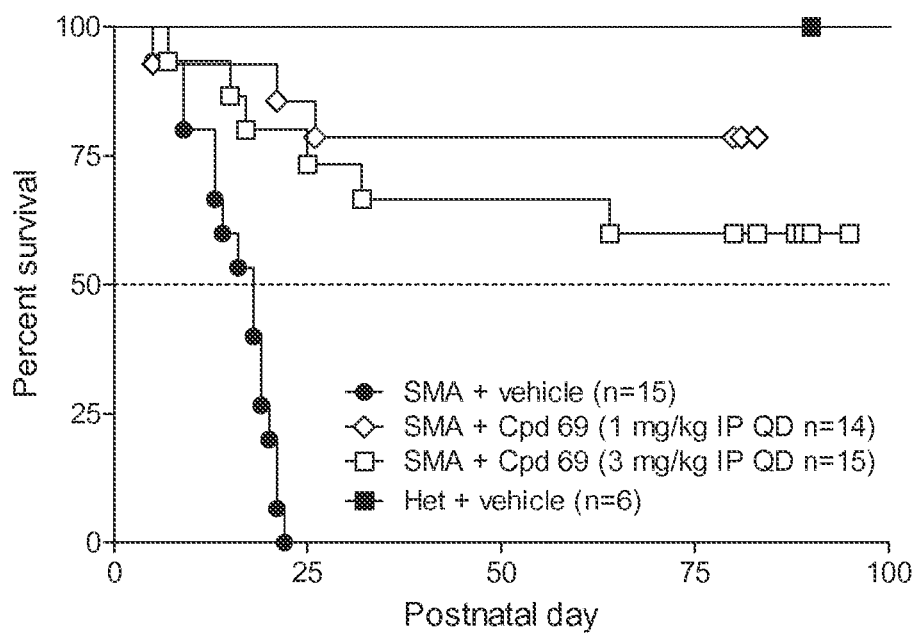


Figure 15b

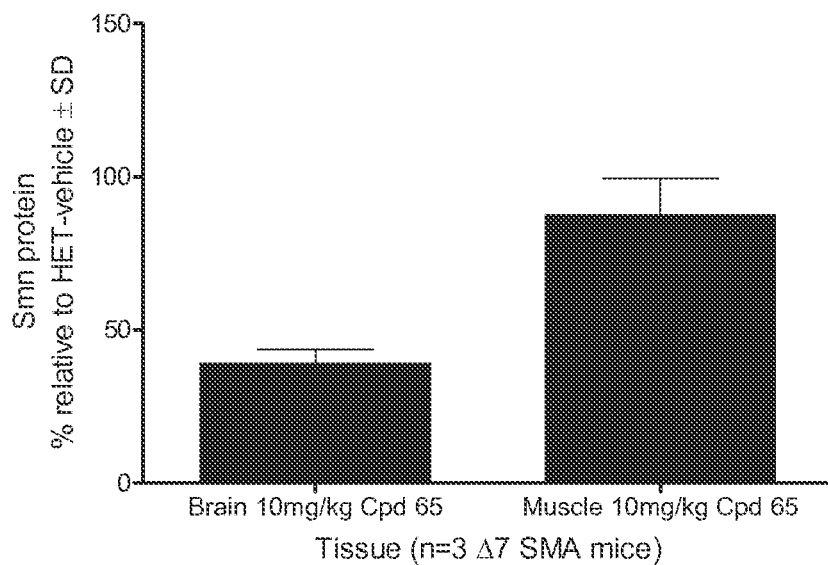


Figure 16a

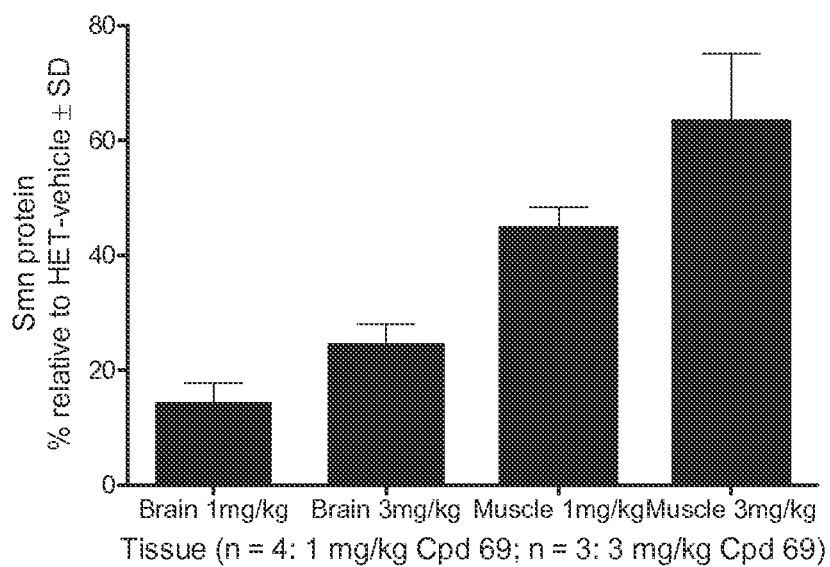


Figure 16b

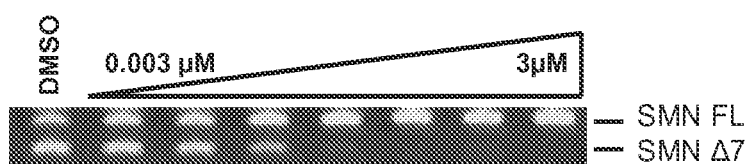


Figure 17a

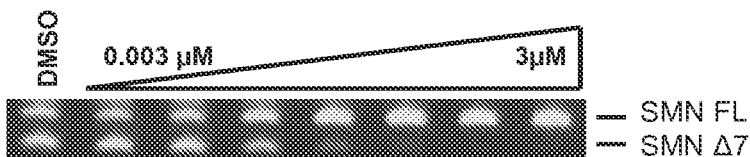


Figure 17b

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COMPOUNDS FOR TREATING SPINAL MUSCULAR ATROPHY

CROSS-REFERENCE

This application is a U.S. national stage application of International Patent Application No. PCT/US2013/023067, filed Jan. 25, 2013, which claims the benefit of priority to U.S. Provisional Application Ser. No. 61/591,102, filed Jan. 26, 2012, which is incorporated herein by reference in its entirety and for all purposes.

The technology described herein has not been made with U.S. Government support.

STATEMENT ON JOINT RESEARCH AGREEMENT

The subject matter disclosed was developed and the claimed invention was made by, or on behalf of, one or more parties to a joint research agreement that was in effect on or before the effective filing date of the claimed invention;

the claimed invention was made as a result of activities undertaken within the scope of the joint research agreement; and

the application for patent for the claimed invention discloses or is amended to disclose the names of the parties to the joint research agreement.

INTRODUCTION

Provided herein are compounds, compositions thereof and uses therewith for treating Spinal Muscular Atrophy.

BACKGROUND

Spinal muscular atrophy (SMA), in its broadest sense, describes a collection of inherited and acquired central nervous system (CNS) diseases characterized by progressive motor neuron loss in the spinal cord and brainstem causing muscle weakness and muscle atrophy. The most common form of SMA is caused by mutations in the Survival Motor Neuron (SMN) gene and manifests over a wide range of severity affecting infants through adults (Crawford and Pardo, *Neurobiol. Dis.*, 1996, 3:97).

Infantile SMA is the most severe form of this neurodegenerative disorder. Symptoms include muscle weakness, poor muscle tone, weak cry, limpness or a tendency to flop, difficulty sucking or swallowing, accumulation of secretions in the lungs or throat, feeding difficulties, and increased susceptibility to respiratory tract infections. The legs tend to be weaker than the arms and developmental milestones, such as lifting the head or sitting up, cannot be reached. In general, the earlier the symptoms appear, the shorter the lifespan. As the motor neuron cells deteriorate, symptoms appear shortly afterward. The severe forms of the disease are fatal and all forms have no known cure. The course of SMA is directly related to the rate of motor neuron cell deterioration and the resulting severity of weakness. Infants with a severe form of SMA frequently succumb to respiratory disease due to weakness in the muscles that support breathing. Children with milder forms of SMA live much longer, although they may need extensive medical support, especially those at the more severe end of the spectrum. The clinical spectrum of SMA disorders has been divided into the following five groups.

(a) Type 0 SMA (In Utero SMA) is the most severe form of the disease and begins before birth. Usually, the first symptom of Type 0 SMA is reduced movement of the fetus that can first

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be observed between 30 and 36 weeks of pregnancy. After birth, these newborns have little movement and have difficulties with swallowing and breathing.

(b) Type 1 SMA (Infantile SMA or Werdnig-Hoffmann disease) typically presents symptoms between 0 and 6 months. This form of SMA is also very severe. Patients never achieve the ability to sit, and death usually occurs within the first 2 years without ventilatory support.

(c) Type 2 SMA (Intermediate SMA) has an age of onset at 7-18 months. Patients achieve the ability to sit unsupported, but never stand or walk unaided. Prognosis in this group is largely dependent on the degree of respiratory involvement.

(d) Type 3 SMA (Juvenile SMA or Kugelberg-Welander disease) is generally diagnosed after 18 months. Type 3 SMA individuals are able to walk independently at some point during their disease course but often become wheelchair-bound during youth or adulthood.

(e) Type 4 SMA (Adult onset SMA). Weakness usually begins in late adolescence in the tongue, hands, or feet, then progresses to other areas of the body. The course of adult SMA is much slower and has little or no impact on life expectancy.

The SMN gene has been mapped by linkage analysis to a complex region in chromosome 5q. In humans, this region contains an approximately 500 thousand base pairs (kb) inverted duplication resulting in two nearly identical copies of the SMN gene. SMA is caused by an inactivating mutation or deletion of the telomeric copy of the gene (SMN1) in both chromosomes, resulting in the loss of SMN1 gene function. However, all patients retain the centromeric copy of the gene (SMN2), and the copy number of the SMN2 gene in SMA patients generally correlates inversely with the disease severity; i.e., patients with less severe SMA have more copies of SMN2. Nevertheless, SMN2 is unable to compensate completely for the loss of SMN1 function due to alternative splicing of exon 7 caused by a translationally silent C to T mutation in exon 7. As a result, the majority of transcripts produced from SMN2 lack exon 7 (SMN2 Δ 7), and encode a truncated Smn protein that has an impaired function and is rapidly degraded.

The Smn protein is thought to play a role in RNA processing and metabolism, having a well characterized function of mediating the assembly of a specific class of RNA-protein complexes termed snRNPs. Smn may have other functions in motor neurons, however its role in preventing the selective degeneration of motor neurons is not well established.

In most cases, SMA is diagnosed based on clinical symptoms and by the absence of all copies of exon 7 in the SMN1 gene, as determined by genetic testing. However, in approximately 5% of cases, SMA is caused by mutations other than a deletion of the entire SMN1 gene or other than a deletion of the entire exon 7 in the SMN1 gene, some known and others not yet defined. In such cases, when the SMN1 gene test is not feasible or the SMN1 gene sequence does not show any abnormality, other tests such as an electromyography (EMG) or muscle biopsy may be indicated.

Medical care for SMA patients at present is limited to supportive therapy including respiratory, nutritional and rehabilitation care; there is no drug known to address the underlying cause of the disease. Current treatment for SMA consists of prevention and management of the secondary effects of chronic motor unit loss. The major management issue in Type 1 SMA is the prevention and early treatment of pulmonary problems, which are the primary cause of death in the majority of the cases. While some infants afflicted with SMA grow to be adults, those with Type 1 SMA have a life expectancy of less than two years.

Several mouse models of SMA have been developed. In particular, the SMN Δ 7 model (Le et al., Hum. Mol. Genet., 2005, 14:845) carries both the SMN2 gene and several copies of the SMN2 Δ 7 cDNA and recapitulates many of the phenotypic features of Type 1 SMA. The SMN Δ 7 model can be used for both SMN2 expression studies as well as the evaluation of motor function and survival. The C/C-allele mouse model (Jackson Laboratory strain No.: 008714) provides a less severe SMA disease model, with mice having reduced levels of both SMN2 full length (SMN2 FL) mRNA and Smn protein. The C/C-allele mouse phenotype has the SMN2 gene and a hybrid mSmn1-SMN2 gene that undergoes alternative splicing, but does not have overt muscle weakness. The C/C-allele mouse model is used for SMN2 expression studies.

As a result of improved understanding of the genetic basis and pathophysiology of SMA, several strategies for treatment have been explored, but none have yet demonstrated success in the clinic.

Gene replacement of SMN1, using viral delivery vectors, and cell replacement, using differentiated SMN1^{+/+} stem cells, have demonstrated efficacy in animal models of SMA. More research is needed to determine the safety and immune response and to address the requirement for the initiation of treatment at the neonatal stage before these approaches can be applied to humans.

Correction of alternative splicing of SMN2 in cultured cells has also been achieved using synthetic nucleic acids as therapeutic agents: (i) antisense oligonucleotides that target sequence elements in SMN2 pre-mRNA and shift the outcome of the splicing reaction toward the generation of full length SMN2 mRNA (Passini et al., Sci. Transl. Med., 2011, 3:72ra18; and, Hua et al., Nature, 2011, 478:123) and (ii) trans-splicing RNA molecules that provide a fully functional RNA sequence that replace the mutant fragment during splicing and generate a full length SMN1 mRNA (Coady and Lorson, J. Neurosci., 2010, 30:126).

Other approaches under exploration include searching for drugs that increase Smn levels, enhance residual Smn function, or compensate for loss of Smn. Aminoglycosides have been shown to enhance expression of stabilized Smn protein produced from SMN2 Δ 7 mRNA by promoting the translational read-through of the aberrant stop codon, but have poor central nervous system penetration and are toxic after repeated dosing. Chemotherapeutic agents, such as aclarubicin, have been shown to increase Smn protein in cell culture; however, the toxicity profile of these drugs prohibits long-term use in SMA patients. Some drugs under clinical investigation for the treatment of SMA include transcription activators such as histone deacetylase ("HDAC") inhibitors (e.g., butyrates, valproic acid, and hydroxyurea), and mRNA stabilizers (mRNA decapping inhibitor RG3039 from Repligen), intended to increase the amount of total RNA transcribed from the SMN2 gene. However, the use of HDAC inhibitors or mRNA stabilizers does not address the underlying cause of SMA and may result in a global increase in transcription and gene expression with potential safety problems in humans.

In an alternative approach, neuroprotective agents such as olesoxime have been chosen for investigation. Such strategies are not aimed at increasing the production of functional Smn for the treatment of SMA, but instead are being explored to protect the Smn-deficient motor neurons from neurodegeneration.

A system designed to identify compounds that increase the inclusion of exon 7 of SMN into RNA transcribed from the SMN2 gene and certain benzoxazole and benzoisoxazole compounds identified thereby have been described in Inter-

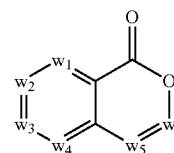
national Application PCT/US2009/003238 filed May 27, 2009 (published as International Publication Number WO2009/151546 and United States Publication Number US2011/0086833). A system designed to identify compounds that produce a stabilized Smn protein from SMN2 Δ 7 mRNA and certain isoindolinone compounds identified thereby have been described in International Application PCT/US2009/004625 filed Aug. 13, 2009 (published as International Publication Number WO2010/019236 and United States Publication Number US2011/0172284). Each of the foregoing documents is herein incorporated in their entirety and for all purposes.

All other documents referred to herein are incorporated by reference into the present application as though fully set forth herein.

Despite the progress made in understanding the genetic basis and pathophysiology of SMA, there remains a need to identify compounds that alter the course of spinal muscular atrophy, one of the most devastating childhood neurological diseases.

SUMMARY

In one aspect, provided herein are compounds of Formula (I):



(I)

or a form thereof, wherein w_1 , w_2 , w_3 , w_4 , w_5 and w_6 are as defined herein. In one embodiment, provided herein is a pharmaceutical composition comprising a compound of Formula (I) or a form thereof, and a pharmaceutically acceptable carrier, excipient or diluent. In a specific embodiment, provided herein is a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof for treating spinal muscular atrophy (SMA).

SMA is caused by deletion or mutation of the SMN1 gene, resulting in selective degeneration of Smn-deficient motor neurons. Although human subjects retain several copies of the SMN2 gene, the small amount of functional Smn protein expressed from SMN2 does not fully compensate for the loss of Smn that would have been expressed from the SMN1 gene. The compounds, compositions thereof and uses therewith described herein are based, in part, on the Applicants discovery that a compound of Formula (I) increases the inclusion of exon 7 of SMN2 into mRNA that is transcribed from an SMN2 minigene. The minigene reproduces the alternative splicing reaction of exon 7 of SMN2 which results in exon 7 skipping in the majority of SMN2 transcripts. Thus, compounds of Formula (I) or a form thereof may be used to modulate inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. Applicants have also discovered that a compound of Formula (I) increases the inclusion of exon 7 of SMN1 into mRNA that is transcribed from an SMN1 minigene. Thus, compounds of Formula (I) or a form thereof may be used to modulate inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene.

In a specific embodiment, provided herein are compounds of Formula (I) or a form thereof that may be used to modulate the inclusion of exon 7 of SMN2 into mRNA that is tran-

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scribed from the SMN2 gene. In another specific embodiment, provided herein are compounds of Formula (I) or a form thereof that may be used to modulate the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene. In yet another embodiment, provided herein are compounds of Formula (I) or a form thereof that may be used to modulate the inclusion of exon 7 of SMN1 and SMN2 into mRNA that is transcribed from the SMN1 and SMN2 genes, respectively.

In another aspect, provided herein is the use of a compound of Formula (I) or a form thereof for treating SMA. In a specific embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject an effective amount of a compound of Formula (I) or a form thereof. The compound of Formula (I) or a form thereof is preferably administered to a human subject in a pharmaceutical composition. In another specific embodiment, provided herein is the use of a compound of Formula (I) for treating SMA, wherein the compound enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. Without being limited by theory, compounds of Formula (I) enhance inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene and increase levels of Smn protein produced from the SMN2 gene, and thus can be used to treat SMA in a human subject in need thereof.

In another aspect, provided herein are primers and/or probes described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13, and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) and the use of those primers and/or probes. In a specific embodiment, provided herein is an isolated nucleotide sequence comprising SEQ ID NO. 1, 2, 3, 7, 8, 9, 10, 11, 12 or 13. In another specific embodiment, provided herein is an isolated nucleotide sequence consisting essentially of SEQ ID NO. 1, 2, 3, 7, 8, 9, 10, 11, 12 or 13. In another specific embodiment, provided herein is an isolated nucleotide sequence consisting of SEQ ID NO. 1, 2, 3, 7, 8, 9, 10, 11, 12 or 13.

In certain embodiments, the amount of mRNA that is transcribed from the SMN1 gene and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 may be used as a biomarker for SMA, such as disclosed herein. In other embodiments, the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 may be used as a biomarker for treating a patient with a compound, such as disclosed herein. In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In certain embodiments, the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 as well as the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 may be used as biomarkers for treating a patient with a compound, such as disclosed herein. In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In accordance with these embodiments, an SMN primer(s) and/or an SMN probe described below may be used in assays, such as PCR (e.g., qPCR), rolling circle amplification, and RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR) to assess and/or quantify the amount of mRNA that is transcribed from the SMN1 gene and/or SMN2 gene and does or does not include exon 7 of SMN1 and/or SMN2.

In a specific embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as

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SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to determine whether a compound (e.g., a compound of Formula (I) or a form thereof) enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from an SMN2 gene.

In a specific embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to determine whether a compound (e.g., a compound of Formula (I) or a form thereof) enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from an SMN1 gene.

In a specific embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to determine whether a compound (e.g., a compound of Formula (I) or a form thereof) enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from an SMN1 and/or SMN2 gene.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 7, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor the amount of mRNA that is transcribed from the SMN2 gene and includes exon 7 of SMN2 in a patient sample. In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 7, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor the amount of mRNA that is transcribed from the SMN1 gene and includes exon 7 of SMN1 in a patient sample. In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 7, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in a patient sample. In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 7, 8, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor a patient's response to a compound (e.g., a compound of Formula (I) or a form thereof). In a specific embodiment, the patient is an SMA patient. In another specific embodiment, the patient is not an SMA patient.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising (a) contacting mRNA that is transcribed from an SMN2 minigene described herein or in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a primer(s) described herein (e.g., SEQ ID NO. 1 and/or 2) along with applicable components for, e.g., RT-PCR, RT-qPCR, PCR, endpoint RT-PCR, qPCR or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene, comprising (a) contacting mRNA that is transcribed from an SMN1 minigene described in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a primer(s) described herein (e.g., SEQ ID NO. 1 and/or 2) along with applicable components for, e.g., RT-PCR, RT-qPCR, PCR, endpoint RT-PCR, qPCR or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN1, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount

of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising (a) contacting mRNA that is transcribed from an SMN2 minigene described herein or in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene, comprising (a) contacting mRNA that is transcribed from an SMN1 minigene described in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN1, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of

SMN2 into mRNA that is transcribed from the SMN2 gene, comprising (a) contacting mRNA that is transcribed from an SMN2 minigene described herein or in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a primer(s) (e.g., SEQ ID NO. 1 or 2) and/or a probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN2 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene.

In another embodiment, provided herein is a method for determining whether a compound (e.g., a compound of Formula (I) disclosed herein) enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene, comprising (a) contacting mRNA that is transcribed from an SMN1 minigene described in International Application PCT/US2009/004625, filed Aug. 13, 2009 (published as International Publication Number WO2010/019236) or United States Publication Number US2011/0172284 in the presence of a compound (e.g., a compound of Formula (I) disclosed herein) with a primer(s) (e.g., SEQ ID NO. 1 or 2) and/or a probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the minigene and includes exon 7 of the SMN1, wherein (1) an increase in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound enhances inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the presence of the compound relative to the amount of mRNA that is transcribed from the minigene and includes exon 7 of SMN1 in the absence of the compound indicates that the compound does not enhance the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene.

In another aspect, provided herein are kits comprising a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) and the use thereof.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1, referenced in Biological Example 1, is a schematic drawing of the SMN2-A minigene construct, which produces

two alternatively spliced mRNA transcripts: a full length mRNA that contains exon 7 and a $\Delta 7$ mRNA that lacks exon 7. The adenine nucleotide inserted in exon 7 of SMN2-A after nucleic residue 48 is represented by the letter "A." Alternatively, the nucleotide may also be selected from cytosine or thymine. Due to the insertion of one nucleotide (A, C, or T) after nucleic residue 48, the full length mRNA does not contain a stop codon in the SMN open reading frame, whereas the $\Delta 7$ mRNA has a stop codon in Exon 8 that is indicated by the word "Stop."

FIG. 2, referenced in Biological Example 1, provides the DNA sequence of the minigene from the SMN2-A minigene construct SEQ ID NO. 21 (FIG. 2a). As shown in FIG. 2b, the following sub-sequences can be found:

1-70: 5'UTR (deg);
71-79: exon 6: start codon and BamHI site (atgggatcc);
80-190: exon 6;
191-5959: intron 6;
5960-6014: exon 7 with the adenine nucleotide "A" insert (position 6008);
6015-6458: intron 7;
6459-6481: part of exon 8;
6482-8146: BamHI site (sequence at the 5' end), luciferase coding sequence starting with codon 2 (without initiation codon), NotI site (sequence at the 3' end), TAA stop codon; and
8147-8266: 3'UTR (deg).

To generate the SMN1 version of the minigene, the sixth nucleotide of exon 7 (a thymine residue) of the SMN2-A minigene construct is changed to cytosine using site directed mutagenesis. Thus, similar to the SMN2-A minigene construct, the SMN1 minigene construct has a single adenine residue inserted after nucleic residue 48 of exon 7. The SMN1 minigene construct is referred to as SMN1-A. Similarly, the nucleotide inserted in the SMN1 minigene construct after nucleic residue 48 of exon 7 may also be selected alternatively from cytosine or thymine.

FIG. 3, referenced in Biological Example 2, shows the correction of SMN2 minigene alternative splicing in cells treated with rising concentrations of Compound 65 (FIG. 3a) and Compound 69 (FIG. 3b) over a 24 hr period. The levels of full length SMN2 minigene mRNA were quantified using reverse transcription-quantitative PCR (RT-qPCR). The level of full length SMN2 minigene mRNA in compound-treated samples was normalized to that in vehicle-treated samples and plotted as a function of the compound concentration.

FIG. 4, referenced in Biological Example 3, shows the correction of SMN2 alternative splicing in Type 1 SMA patient fibroblasts treated with rising concentrations of Compound 65 (FIG. 4a) and Compound 69 (FIG. 4b) over a 24 hr period. The levels of full length and $\Delta 7$ SMN2 mRNA were quantified using RT-qPCR. The levels of full length and $\Delta 7$ SMN2 mRNA in compound-treated samples were normalized to those in vehicle-treated samples and plotted as a function of the compound concentration.

FIG. 5, referenced in Biological Example 4, shows the correction of SMN2 alternative splicing in Type 1 SMA patient fibroblasts treated with rising concentrations of Compound 65 (FIG. 5a) and Compound 69 (FIG. 5b) over a 24 hr period. The full length and $\Delta 7$ SMN2 mRNA were amplified using reverse transcription-end point PCR (RT-PCR) and PCR products were separated using agarose gel electrophoresis. The top and bottom bands correspond to the full length and $\Delta 7$ SMN2 mRNA respectively. The intensity of each band is proportional to the amount of RNA present in the sample.

FIG. 6, referenced in Biological Example 5, shows the correction of SMN2 alternative splicing (in both the SMN2

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gene and the hybrid mouse *Smn1-SMN2* gene) in brain and muscle tissues in a C/C-allele SMA mouse model resulting from treatment for 10 days twice per day (BID) with 10 mg/kg of Compound 65 (FIG. 6a) and Compound 69 (FIG. 6b). The levels of full length and $\Delta 7$ SMN2 mRNA were quantified using RT-qPCR, the combined full length and $\Delta 7$ SMN2 mRNA quantity was set to 1, and fractional quantities of full length and $\Delta 7$ SMN2 were calculated.

FIG. 7, referenced in Biological Example 6, shows the correction of SMN2 alternative splicing (in both the SMN2 gene and the hybrid mouse *Smn1-SMN2* gene) in brain and muscle tissues in a C/C-allele SMA mouse model resulting from treatment for 10 days BID with 10 mg/kg of Compound 65 (FIG. 7). The full length and $\Delta 7$ SMN2 mRNA were amplified using RT-PCR. The PCR products were separated using agarose gel electrophoresis. The top and bottom bands correspond to the full length and $\Delta 7$ SMN2 mRNA, respectively. The intensity of each band is proportional to the amount of RNA present in the sample.

FIG. 8, referenced in Biological Example 7, shows a dose dependent increase in Smn protein expression in SMA Type 1 human fibroblast cells treated over a 48 hour period with Compound 65 (FIG. 8a) and Compound 69 (FIG. 8b).

FIG. 9, referenced in Biological Example 8, shows an increase in nuclear speckle counts (gems) in Type 1 SMA patient fibroblasts treated with Compound 65 (FIG. 9a) and Compound 69 (FIG. 9b) over a 48 hour period. Speckles were counted using fluorescence microscopy. The number of speckles in compound-treated samples was normalized to that in vehicle-treated samples and plotted as a function of the compound concentration.

FIG. 10, referenced in Biological Example 9, shows an increase in Smn protein expression (black circles) in motor neurons generated from iPS cells generated from Type 1 SMA patient fibroblasts treated with Compound 65 (FIG. 10a) and Compound 69 (FIG. 10b) over a 72 hour period. The level of Smn protein was quantified using Smn immunostaining and confocal fluorescence microscopy. The level of Smn protein in compound-treated samples was normalized to that in vehicle-treated samples and plotted as a function of the compound concentration.

FIG. 11, referenced in Biological Example 11, shows increased Smn protein expression in brain, spinal cord, muscle and liver tissues in a C/C-allele SMA mouse model resulting from treatment for 10 days BID with 10 mg/kg of Compound 65 (FIG. 11a, n=5) and Compound 69 (FIG. 11b, n=4), where three stars (***) in each Figure represents $p < 0.001$ by ANOVA.

FIG. 12, referenced in Biological Example 12, shows a dose dependent increase in Smn protein expression in tissues (Brain, FIG. 12a; Spinal cord, FIG. 12b; and Muscle, FIG. 12c) in a neonatal $\Delta 7$ SMA mouse model resulting from treatment for 7 days once per day (QD) with indicated doses of Compound 65, where three stars (***) in each Figure represents $p < 0.001$ by ANOVA.

FIG. 13, referenced in Biological Example 13, shows differences in body weight in a neonatal $\Delta 7$ SMA mouse model resulting from treatment upto Postnatal Day (PND) 140 with Compound 65 (FIG. 13a) and until PND 79 with Compound 69 (FIG. 13b).

FIG. 14, referenced in Biological Example 14, shows an improved righting reflex in a neonatal $\Delta 7$ SMA mouse model resulting from treatment with Compound 65 (FIG. 14a) and Compound 69 (FIG. 14b).

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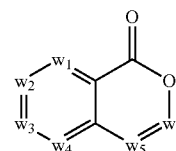
FIG. 15, referenced in Biological Example 15, shows improved survival in a neonatal $\Delta 7$ SMA mouse model resulting from treatment with Compound 65 (FIG. 15a) and Compound 69 (FIG. 15b).

FIG. 16, referenced in Biological Example 15, shows increased Smn protein expression in brain and muscle tissues in a $\Delta 7$ SMA mouse model resulting from treatment with Compound 65 until PND 144 (FIG. 16a) and with Compound 69 until PND 80 and PND 83 (FIG. 16b) relative to vehicle treated and age-matched heterozygous mice, respectively.

FIG. 17, referenced in Biological Example 16, shows a dose dependent increase in SMN1 minigene FL mRNA and a dose dependent decrease in SMN1 minigene $\Delta 7$ mRNA in SMA Type 1 human fibroblast cells treated over a 7 hour period with Compound 65 (FIG. 17a) and Compound 69 (FIG. 17b). The full length and $\Delta 7$ SMN1 minigene mRNA were each amplified using RT-PCR and the resulting PCR products were separated using agarose gel electrophoresis. The top and bottom bands correspond to the full length and $\Delta 7$ SMN1 minigene mRNA, respectively. The intensity of each band is proportional to the amount of RNA present in the sample.

DETAILED DESCRIPTION

Provided herein are compounds of Formula (I):



(I)

or a form thereof, wherein:

w_1 is C— R_b or N;

w_2 and w_6 are C— R_1 or C— R_2 ;

w_3 , w_4 and w_5 are C— R_a or N;

wherein one of w_2 and w_6 is C— R_1 and the other is C— R_2 , provided that, when w_2 is C— R_1 , then w_6 is C— R_2 ; or, when w_2 is C— R_2 , then w_6 is C— R_1 ; and,

wherein any one, two or three of the remaining w_1 , w_3 , w_4 and w_5 may simultaneously be N;

R_1 is C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, amino-C₁₋₈alkyl-amino, (amino-C₁₋₈alkyl)₂-amino, (amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, C₁₋₈alkyl-amino-C₁₋₈alkoxy, (C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, amino-C₂₋₈alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl, amino-C₂₋₈alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkynyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkynyl, halo-C₁₋₈alkyl-amino, (halo-C₁₋₈alkyl)₂-amino, (halo-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl,

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hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl-amino, [(hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, [(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, heterocyclyl, heterocyclyl-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkoxy, heterocyclyl-amino, (heterocyclyl)(C₁₋₈alkyl)amino, heterocyclyl-amino-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkyl-amino, (heterocyclyl-C₁₋₈alkyl)₂-amino, (heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, heterocyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (heterocyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heterocyclyl-oxy, heterocyclyl-carbonyl, heterocyclyl-carbonyl-oxy, aryl-C₁₋₈alkyl-amino, (aryl-C₁₋₈alkyl)₂-amino, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heteroaryl, hetero aryl-C₁₋₈alkyl, heteroaryl-C₁₋₈alkoxy, heteroaryl-amino, heteroaryl-C₁₋₈alkyl-amino, (heteroaryl-C₁₋₈alkyl)₂-amino, (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hetero aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hetero aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl or (hetero aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl; wherein, each instance of heterocyclyl and heteroaryl is optionally substituted with one, two or three R₃ substituents and one additional, optional R₄ substituent; and, wherein, alternatively, each instance of heterocyclyl and heteroaryl is optionally substituted with one, two, three or four R₃ substituents; R₂ is aryl, aryl-amino, aryl-amino-carbonyl, heterocyclyl, heteroaryl or heteroaryl-amino; wherein, each instance of aryl, heterocyclyl and heteroaryl is optionally substituted with one, two or three R₆ substituents and one additional, optional R₇ substituent; R_a is, in each instance, independently selected from hydrogen, halogen or C₁₋₈alkyl; R_b is hydrogen, halogen, C₁₋₈alkyl or C₁₋₈alkoxy; R₃ is, in each instance, independently selected from cyano, halogen, hydroxy, oxo, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkyl-carbonyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, C₁₋₈alkoxy-carbonyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, amino-C₁₋₈alkyl-amino, C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl]₂-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-carbonyl-amino, C₁₋₈alkoxy-carbonyl-amino, hydroxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino or (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino; R₄ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-C₁₋₈alkyl, C₃₋₁₄cycloalkyl-amino, aryl-C₁₋₈alkyl, aryl-C₁₋₈alkoxy-carbonyl, aryl-sulfonyloxy-C₁₋₈alkyl, heterocyclyl or hetero-

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cyclyl-C₁₋₈alkyl; wherein, each instance of C₃₋₁₄cycloalkyl, aryl and heterocyclyl is optionally substituted with one, two or three R₅ substituents; R₅ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio; R₆ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, C₂₋₈alkenyl, halo-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio; and, R₇ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-oxy, aryl, heterocyclyl or heteroaryl.

EMBODIMENTS

In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, one of w₂ and w₆ is C—R₁ and the other is C—R₂, provided that, when w₂ is C—R₁, then w₆ is C—R₂; or, when w₂ is C—R₂, then w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₃ is C—R_a; w₄ is N; w₅ is C—R_a; and, one of w₂ and w₆ is C—R₁ and the other is C—R₂, provided that, when w₂ is C—R₁, then w₆ is C—R₂; or, when w₂ is C—R₂, then w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₃ is N; w₄ is C—R_a; w₅ is C—R_a; and, one of w₂ and w₆ is C—R₁ and the other is C—R₂, provided that, when w₂ is C—R₁, then w₆ is C—R₂; or, when w₂ is C—R₂, then w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is N; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, one of w₂ and w₆ is C—R₁ and the other is C—R₂, provided that, when w₂ is C—R₁, then w₆ is C—R₂; or, when w₂ is C—R₂, then w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₃ is C—R_a; w₄ is C—R_a; w₅ is N; and, one of w₂ and w₆ is C—R₁ and the other is C—R₂, provided that, when w₂ is C—R₁, then w₆ is C—R₂; or, when w₂ is C—R₂, then w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₂ is C—R₁; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₂. In another embodiment of a compound of Formula (I), w₁ is C—R_b; w₂ is C—R₂; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is C—R_b; w₂ is C—R₁; w₃ is N; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₂. In another embodiment of a compound of Formula (I), w₁ is C—R_b; w₂ is C—R₂; w₃ is N; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₁. In one embodiment of a compound of Formula (I), w₁ is N; w₂ is C—R₁; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₂. In another embodiment of a compound of Formula (I), w₁ is N; w₂ is C—R₂; w₃ is C—R_a; w₄ is C—R_a; w₅ is C—R_a; and, w₆ is C—R₁.

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In one embodiment of a compound of Formula (I), w_1 is $C-R_b$; w_2 is $C-R_1$; w_3 is $C-R_a$; w_4 is $C-R_a$; w_5 is N; and, w_6 is $C-R_2$.

In another embodiment of a compound of Formula (I), w_1 is C—R_b; w_2 is C—R₂; w_3 is C—R_a; w_4 is C—R_a; w_5 is N; and, w_6 is C—R₁.

In one embodiment of a compound of Formula (I), w_1 is $C-R_b$.

In another embodiment of a compound of Formula (I), w_1 is N.

In one embodiment of a compound of Formula (I), w_2 is $C-R_1$, provided that w_6 is $C-R_2$.

In another embodiment of a compound of Formula (I), w_2 is $C-R_2$, provided that w_6 is $C-R_1$.

In one embodiment of a compound of Formula (I), w_6 is $C-R_1$, provided that w_2 is $C-R_2$.

In another embodiment of a compound of Formula (I), w_6 is $C-R_2$, provided that w_2 is $C-R_1$.

In one embodiment of a compound of Formula (I), w_3 is C—R_g. 20

In another embodiment of a compound of Formula (I), Av₃ is N.

In one embodiment of a compound of Formula (I), w_4 is C—R₉.

In another embodiment of a compound of Formula (I), w₄ is N.

In one embodiment of a compound of Formula (I), w_5 is $C-R_a$.

In another embodiment of a compound of Formula (I), w_5 is N.

In one embodiment of a compound of Formula (I),

R₁ is C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl) amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, 35

(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, amino-C₁₋₈alkyl-amino, (amino-C₁₋₈alkyl)₂-

amino, (amino- C_{1-8} alkyl)(C_{1-8} alkyl)amino, C_{1-8} alkyl-40
amino- C_{1-8} alkyl-amino, (C_{1-8} alkyl-amino- C_{1-8} alkyl)₂-
amino, (C_{1-8} alkyl-amino- C_{1-8} alkyl)(C_{1-8} alkyl)amino,
(C_{1-8} alkyl)₂-amino- C_{1-8} alkyl-amino, [(C_{1-8} alkyl)₂-
amino- C_{1-8} alkyl](C_{1-8} alkyl)amino, amino- C_{1-8} alkoxy-

amino-C₁₋₈alkyl]-(C₁₋₈alkyl)amino-, amino-C₁₋₈alkoxy-,
C₁₋₈alkyl-amino-C₁₋₈alkoxy-, (C₁₋₈alkyl)₂-amino-C₁₋₈ 45
alkoxy-, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy-, (C₁₋₈
alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy-, (C₁₋₈alkoxy-
C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy-, amino-C₁₋₈

alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl, amino-C₂₋₈alkynyl, C₁₋₈alkyl-amino-C₂₋₈alkynyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkynyl, (C₁₋₈alkyl)-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl, halo-C₁₋₈ alkyl-amino, (halo-C₁₋₈alkyl)-amino, (halo-

C₁₋₈ alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl,
hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-
amino, (hydroxy-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈ 55
alkyl)(C₁₋₈ alkyl)amino, hydroxy-C₁₋₈alkyl-amino-C₁₋₈

alkyl, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl) 60

amino-C₁₋₈ alkoxy, hydroxy-C₁₋₈alkyl-amino-C₁₋₈
alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-
amino, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino,
(hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino,
(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl amino,

amino, [(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl]

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alkyl)(C₁₋₈alkyl)amino, heterocyclyl, heterocyclyl-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkoxy, heterocyclyl-amino, (hetero cyclyl)(C₁₋₈alkyl)amino, hetero cyclyl-amino-C₁₋₈alkyl, hetero cyclyl-C₁₋₈alkyl-amino, (hetero cyclyl-C₁₋₈alkyl)₂-amino, (hetero cyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hetero cyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hetero cyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hetero cyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heterocyclyl-oxy, heterocyclyl-carbonyl, heterocyclyl-carbonyl-oxy, aryl-C₁₋₈alkyl-amino, (aryl-C₁₋₈alkyl)₂-amino, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heteroaryl, hetero aryl-C₁₋₈alkyl, hetero aryl-C₁₋₈alkoxy, hetero aryl-amino, hetero aryl-C₁₋₈alkyl-amino, (hetero aryl-C₁₋₈alkyl)₂-amino, (hetero aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hetero aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hetero aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl or (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl; wherein, each instance of heterocyclyl and heteroaryl is optionally substituted with R₁ and R₄ substituents.

In another embodiment of a compound of Formula (I),

R₁ is amino, (C₁₋₈alkyl)₂-amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, amino-C₁₋₈alkyl-amino, (amino-C₁₋₈alkyl)₂-amino, (amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, amino-C₁₋₈alkoxy, C₁₋₈alkyl-amino-C₁₋₈alkoxy, (C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, amino-C₂₋₈alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl, amino-C₂₋₈alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl, halo-C₁₋₈alkyl-amino, (halo-C₁₋₈alkyl)₂-amino, (halo-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl-amino, [(hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, [(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, heterocyclyl, heterocyclyl-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkoxy, heterocyclyl-amino, (heterocyclyl)(C₁₋₈alkyl)amino, hetero cyclyl-amino-C₁₋₈alkyl, hetero cyclyl-C₁₋₈alkyl-amino, (hetero cyclyl-C₁₋₈alkyl)₂-amino, (hetero cyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hetero cyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hetero cyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hetero cyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heterocycl-oxo, heterocyclyl-carbonyl, heterocyclyl-carbonyl-oxo, aryl-C₁₋₈alkyl-amino, (aryl-C₁₋₈alkyl)₂-amino,

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(aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heteroaryl, heteroaryl-C₁₋₈alkyl, heteroaryl-C₁₋₈alkoxy, heteroaryl-C₁₋₈alkyl-amino, (heteroaryl-C₁₋₈alkyl)₂-amino, (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, heteroaryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (heteroaryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl or (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl; wherein, each instance of heterocyclyl and heteroaryl is optionally substituted with R₃ and R₄ substituents.

In one embodiment of a compound of Formula (I), R₁ is heterocyclyl selected from azetidiny, tetrahydrofuranyl, pyrrolidinyl, piperidinyl, piperazinyl, 1,4-diazepanyl, 1,2,5,6-tetrahydropyridinyl, 1,2,3,6-tetrahydropyridinyl, hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, (3aS,6aS)-hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, hexahydropyrrolo[3,4-b]pyrrol-(2H)-yl, (3aS,6aS)-hexahydropyrrolo[3,4-b]pyrrol-(2H)-yl, hexahydropyrrolo[3,4-c]pyrrol-(1H)-yl, (3aR,6aS)-hexahydropyrrolo[3,4-c]pyrrol-(1H)-yl, octahydro-5H-pyrrolo[3,2-c]pyridinyl, octahydro-6H-pyrrolo[3,4-b]pyridinyl, (4aR,7aR)-octahydro-6H-pyrrolo[3,4-b]pyridinyl, (4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridinyl, hexahydropyrrolo[1,2-a]pyrazin-(2H)-one, hexahydropyrrolo[1,2-a]pyrazin-(1H)-yl, (7R,8aS)-hexahydropyrrolo[1,2-a]pyrazin-(1H)-yl, (8aS)-hexahydropyrrolo[1,2-a]pyrazin-(1H)-yl, (8aR)-hexahydropyrrolo[1,2-a]pyrazin-(1H)-yl, (8aS)-octahydropyrrolo[1,2-a]pyrazin-(1H)-yl, (8aR)-octahydropyrrolo[1,2-a]pyrazin-(1H)-yl, octahydro-2H-pyrido[1,2-a]pyrazinyl, 3-azabicyclo[3.1.0]hexyl, (1R,5S)-3-azabicyclo[3.1.0]hexyl, 8-azabicyclo[3.2.1]octyl, (1R,5S)-8-azabicyclo[3.2.1]octyl, 8-azabicyclo[3.2.1]oct-2-enyl, (1R,5S)-8-azabicyclo[3.2.1]oct-2-enyl, 9-azabicyclo[3.3.1]nonyl, (1R,5S)-9-azabicyclo[3.3.1]nonyl, 2,5-diazabicyclo[2.2.1]heptyl, (1S,4S)-2,5-diazabicyclo[2.2.1]heptyl, 2,5-diazabicyclo[2.2.2]octyl, 3,8-diazabicyclo[3.2.1]octyl, (1R,5S)-3,8-diazabicyclo[3.2.1]octyl, 1,4-diazabicyclo[3.2.2]nonyl, azaspiro[3.3]heptyl, 2,6-diazaspiro[3.3]heptyl, 2,7-diazaspiro[3.5]nonyl, 5,8-diazaspiro[3.5]nonyl, 2,7-diazaspiro[4.4]nonyl or 6,9-diazaspiro[4.5]decyl; wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In another embodiment of a compound of Formula (I), R₁ is heterocyclyl selected from azetidin-1-yl, tetrahydrofuran-3-yl, pyrrolidin-1-yl, piperidin-1-yl, piperidin-4-yl, piperazin-1-yl, 1,4-diazepan-1-yl, 1,2,5,6-tetrahydropyridin-5-yl, 1,2,3,6-tetrahydropyridin-4-yl, hexahydropyrrolo[3,4-b]pyrrol-1(2H)-yl, (3aS,6aS)-hexahydropyrrolo[3,4-b]pyrrol-1(2H)-yl, (3aS,6aS)-hexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl, hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, (3aR,6aS)-hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, octahydro-5H-pyrrolo[3,2-c]pyridin-5-yl, octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aR,7aR)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aS,7aS)-octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, hexahydropyrrolo[1,2-a]pyrazin-6(2H)-one, hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, (7R,8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, (8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, (8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, (8aS)-octahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, (8aR)-octahydropyrrolo[1,2-a]pyrazin-2(1H)-yl, octahydro-2H-pyrido[1,2-a]pyrazin-2-yl, 3-azabicyclo[3.1.0]hex-3-yl, 8-azabicyclo[3.2.1]oct-3-yl, (1R,5S)-8-azabicyclo[3.2.1]oct-3-yl, 8-azabicyclo[3.2.1]oct-2-en-3-yl, (1R,5S)-8-azabicyclo[3.2.1]oct-2-en-3-yl, 9-azabicyclo[3.3.1]non-3-yl, (1R,5S)-9-azabicyclo[3.3.1]non-3-yl, 2,5-diazabicyclo[2.2.1]

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hept-2-yl, (1S,4S)-2,5-diazabicyclo[2.2.1]hept-2-yl, 2,5-diazabicyclo[2.2.2]oct-2-yl, 3,8-diazabicyclo[3.2.1]oct-3-yl, (1R,5S)-3,8-diazabicyclo[3.2.1]oct-3-yl, 1,4-diazabicyclo[3.2.2]non-4-yl, azaspiro[3.3]hept-2-yl, 2,6-diazaspiro[3.3]hept-2-yl, 2,7-diazaspiro[3.5]non-7-yl, 5,8-diazaspiro[3.5]non-8-yl, 2,7-diazaspiro[4.4]non-2-yl or 6,9-diazaspiro[4.5]dec-9-yl; wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In another embodiment of a compound of Formula (I), R₁ is substituted heterocyclyl selected from 4-methyl-1,4-diazepan-1-yl, (3aS,6aS)-1-methylhexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl, (3aS,6aS)-5-methylhexahydropyrrolo[3,4-b]pyrrol-1(2H)-yl, (3aR,6aR)-1-methylhexahydropyrrolo[3,4-b]pyrrol-5(1H)-yl, (3aR,6aS)-5-methylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, (3aR,6aS)-5-(2-hydroxyethyl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, (3aR,6aS)-5-(propan-2-yl)hexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, (3aR,6aS)-5-ethylhexahydropyrrolo[3,4-c]pyrrol-2(1H)-yl, (4aR,7aR)-1-methyloctahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aR,7aR)-1-ethyloctahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aR,7aR)-1-(2-hydroxyethyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aS,7aS)-1-methyloctahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (4aS,7aS)-1-(2-hydroxyethyl)octahydro-6H-pyrrolo[3,4-b]pyridin-6-yl, (7R,8aS)-7-hydroxyhexahydropyrrolo[1,2-c]pyrazin-2(1H)-yl, (8aS)-8a-methyloctahydropyrrolo[1,2-c]pyrazin-2(1H)-yl, (8aR)-8a-methyloctahydropyrrolo[1,2-c]pyrazin-2(1H)-yl, (1R,5S,6s)-6-(dimethylamino)-3-azabicyclo[3.1.0]hex-3-yl, (1R,5S)-8-methyl-8-azabicyclo[3.2.1]oct-3-yl, 9-methyl-9-azabicyclo[3.3.1]non-3-yl, (3-exo)-9-methyl-9-azabicyclo[3.3.1]non-3-yl, (1R,5S)-9-methyl-9-azabicyclo[3.3.1]non-3-yl, (1S,4S)-5-methyl-2,5-diazabicyclo[2.2.1]hept-2-yl or (1S,4S)-5-ethyl-2,5-diazabicyclo[2.2.1]hept-2-yl.

In one embodiment of a compound of Formula (I), R₁ is heterocyclyl-C₁₋₈alkyl, wherein heterocyclyl is selected from morpholinyl, piperidinyl, piperazinyl, imidazolyl or pyrrolidinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In another embodiment of a compound of Formula (I), R₁ is heterocyclyl-C₁₋₈alkyl selected from morpholin-4-yl-methyl, morpholin-4-yl-ethyl, morpholin-4-yl-propyl, piperidin-1-yl-methyl, piperazin-1-yl-methyl, piperazin-1-yl-ethyl, piperazin-1-yl-propyl, piperazin-1-yl-butyl, imidazol-1-yl-methyl, imidazol-1-yl-ethyl, imidazol-1-yl-propyl, imidazol-1-yl-butyl, pyrrolidin-1-yl-methyl, pyrrolidin-1-yl-ethyl, pyrrolidin-1-yl-propyl or pyrrolidin-1-yl-butyl; wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In one embodiment of a compound of Formula (I), R₁ is heterocyclyl-C₁₋₈alkoxy, wherein heterocyclyl is selected from pyrrolidinyl, piperidinyl or morpholinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In another embodiment of a compound of Formula (I), R₁ is heterocyclyl-C₁₋₈alkoxy selected from pyrrolidin-2-yl-methoxy, pyrrolidin-2-yl-ethoxy, pyrrolidin-1-yl-methoxy, pyrrolidin-1-yl-ethoxy, piperidin-1-yl-methoxy, piperidin-1-yl-ethoxy, morpholin-4-yl-methoxy or morpholin-4-yl-ethoxy; wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In one embodiment of a compound of Formula (I), R₁ is heterocyclyl-amino, wherein heterocyclyl is selected from azetidiny, pyrrolidinyl, piperidinyl, 9-azabicyclo[3.3.1]nonyl or (1R,5S)-9-azabicyclo[3.3.1]nonyl; and, wherein, each instance of heterocyclyl is optionally substituted with R₃ and R₄ substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl-amino selected from azetidin-3-yl-amino, pyrrolidin-3-yl-amino, piperidin-4-yl-amino, 9-azabicyclo[3.3.1]non-3-yl-amino, (1R,5S)-9-azabicyclo[3.3.1]non-3-yl-amino, 9-methyl-9-azabicyclo[3.3.1]non-3-yl-amino, (3-exo)-9-methyl-9-azabicyclo[3.3.1]non-3-yl-amino or (1R,5S)-9-methyl-9-azabicyclo[3.3.1]non-3-yl-amino; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is (heterocyclyl)(C_{1-8} alkyl)amino, wherein heterocyclyl is selected from pyrrolidinyl or piperidinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is (heterocyclyl)(C_{1-8} alkyl)amino selected from (pyrrolidin-3-yl)(methyl)amino or (piperidin-4-yl)(methyl)amino; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heterocyclyl-amino- C_{1-8} alkyl, wherein heterocyclyl is selected from tetrahydrofuranyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl-amino- C_{1-8} alkyl, selected from 3-(tetrahydrofuran-3-yl-amino)propyl; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heterocyclyl- C_{1-8} alkyl-amino- C_{1-8} alkyl, wherein heterocyclyl is selected from tetrahydrofuranyl, thienyl or pyridinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl- C_{1-8} alkyl-amino- C_{1-8} alkyl, selected from 3-[(tetrahydrofuran-2-ylmethyl)amino]propyl, 3-[(thiophenyl-3-ylmethyl)amino]propyl, 3-[(pyridin-2-ylmethyl)amino]propyl or 3-[(pyridin-4-ylmethyl)amino]propyl; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heterocyclyl-oxy, wherein heterocyclyl is selected from pyrrolidinyl or piperidinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl-oxy selected from pyrrolidin-3-yl-oxy or piperidin-4-yl-oxy; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heterocyclyl-carbonyl, wherein heterocyclyl is selected from piperazinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl-carbonyl selected from piperazin-1-yl-carbonyl; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heterocyclyl-carbonyl-oxy, wherein heterocyclyl is selected from piperazinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heterocyclyl-carbonyl-oxy selected from piperazin-1-yl-carbonyl-oxy; wherein, each instance of heterocyclyl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is aryl- C_{1-8} alkyl-amino- C_{1-8} alkyl, wherein aryl is selected from phenyl; and, wherein, each instance of aryl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is aryl- C_{1-8} alkyl-amino- C_{1-8} alkyl selected from 3-(benzylamino)propyl; wherein, each instance of aryl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heteroaryl, wherein heteroaryl is selected from pyridinyl; and, wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heteroaryl selected from pyridin-4-yl; wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heteroaryl- C_{1-8} alkyl, wherein heteroaryl is selected from 1H-imidazolyl; and, wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heteroaryl- C_{1-8} alkyl selected from 1H-imidazol-1-yl-methyl; wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is (heteroaryl- C_{1-8} alkyl)(C_{1-8} alkyl)amino, wherein heteroaryl is selected from pyridinyl; and, wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is (heteroaryl- C_{1-8} alkyl)(C_{1-8} alkyl)amino selected from (pyridin-3-ylmethyl)(methyl)amino; wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_1 is heteroaryl- C_{1-8} alkyl-amino- C_{1-8} alkyl, wherein heteroaryl is selected from thienyl or pyridinyl; and, wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In another embodiment of a compound of Formula (I), R_1 is heteroaryl- C_{1-8} alkyl-amino- C_{1-8} alkyl selected from thien-3-yl-methyl-amino-propyl, pyridin-2-yl-methyl-amino-propyl, pyridin-3-yl-methyl-amino-propyl or pyridin-4-yl-methyl-amino-propyl; wherein, each instance of heteroaryl is optionally substituted with R_3 and R_4 substituents.

In one embodiment of a compound of Formula (I), R_3 is selected from cyano, halogen, hydroxy, oxo, C_{1-8} alkyl, halo- C_{1-8} alkyl, C_{1-8} alkyl-carbonyl, C_{1-8} alkoxy, halo- C_{1-8} alkoxy, C_{1-8} alkoxy- C_{1-8} alkyl, C_{1-8} alkoxy-carbonyl, amino, C_{1-8} alkyl-amino, (C_{1-8} alkyl)₂-amino, amino- C_{1-8} alkyl, C_{1-8} alkyl-amino- C_{1-8} alkyl, (C_{1-8} alkyl)₂-amino- C_{1-8} alkyl, amino- C_{1-8} alkyl-amino, C_{1-8} alkyl-amino- C_{1-8} alkyl-amino, (C_{1-8} alkyl)₂-amino- C_{1-8} alkyl-amino, C_{1-8} alkoxy- C_{1-8} alkyl-amino, C_{1-8} alkyl-carbonyl-amino, C_{1-8} alkoxy-carbonyl-amino, hydroxy- C_{1-8} alkyl, hydroxy- C_{1-8} alkoxy- C_{1-8} alkyl, hydroxy- C_{1-8} alkyl-amino, (hydroxy- C_{1-8} alkyl)₂-amino or (hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl)amino.

In another embodiment of a compound of Formula (I), R_3 is selected from cyano, halogen, hydroxy, oxo, C_{1-8} alkyl, halo- C_{1-8} alkyl, C_{1-8} alkoxy, C_{1-8} alkoxy- C_{1-8} alkyl, C_{1-8} alkoxy-carbonyl, amino, C_{1-8} alkyl-amino, (C_{1-8} alkyl)₂-amino, amino- C_{1-8} alkyl, C_{1-8} alkyl-amino- C_{1-8} alkyl, (C_{1-8} alkyl)₂-amino- C_{1-8} alkyl, C_{1-8} alkyl-amino- C_{1-8} alkyl-amino, C_{1-8} alkoxy- C_{1-8} alkyl-amino, C_{1-8} alkoxy-carbonyl-amino, hydroxy- C_{1-8} alkyl, hydroxy- C_{1-8} alkoxy- C_{1-8} alkyl, hydroxy- C_{1-8} alkyl-amino, (hydroxy- C_{1-8} alkyl)₂-amino or (hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl)amino.

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In one embodiment of a compound of Formula (I), R₃ is C₁₋₈alkyl selected from methyl, ethyl, propyl, isopropyl or tert-butyl.

In another embodiment of a compound of Formula (I), R₃ is C₁₋₈alkyl selected from ethyl, propyl, isopropyl or tert-butyl.

In one embodiment of a compound of Formula (I), R₃ is halo-C₁₋₈alkyl selected from trihalo-methyl, dihalo-methyl, halo-methyl, trihalo-ethyl, dihalo-ethyl, halo-ethyl, trihalo-propyl, dihalo-propyl or halo-propyl; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R₃ is halo-C₁₋₈alkyl selected from trihalo-methyl, dihalo-methyl, halo-methyl, trihalo-ethyl, dihalo-ethyl, trihalo-propyl or dihalo-propyl; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R₃ is hydroxy-C₁₋₈alkyl selected from hydroxy-methyl, hydroxy-ethyl, hydroxy-propyl, dihydroxy-propyl, hydroxy-butyl or dihydroxy-butyl.

In another embodiment of a compound of Formula (I), R₃ is hydroxy-C₁₋₈alkyl selected from hydroxy-methyl, dihydroxy-propyl, hydroxy-butyl or dihydroxy-butyl.

In one embodiment of a compound of Formula (I), R₃ is C₁₋₈alkoxy selected from methoxy, ethoxy, propoxy or isopropoxy.

In one embodiment of a compound of Formula (I), R₃ is halo-C₁₋₈alkoxy selected from trihalo-methoxy, dihalo-methoxy, halo-methoxy, trihalo-ethoxy, dihalo-ethoxy, halo-ethoxy, trihalo-propoxy, dihalo-propoxy or halo-propoxy; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R₃ is C₁₋₈alkoxy-carbonyl-amino selected from methoxy-carbonyl-amino, ethoxy-carbonyl-amino, propoxy-carbonyl-amino, isopropoxy-carbonyl-amino, tert-butoxy-carbonyl-amino.

In one embodiment of a compound of Formula (I), R₄ is C₃₋₁₄cycloalkyl selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; wherein, each instance of C₃₋₁₄cycloalkyl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is C₃₋₈cycloalkyl selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; wherein, each instance of C₃₋₈cycloalkyl is optionally substituted with R₅ substituents.

In one embodiment of a compound of Formula (I), R₄ is C₃₋₁₄cycloalkyl-C₁₋₈alkyl, wherein C₃₋₁₄cycloalkyl is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; and, wherein, each instance of C₃₋₁₄cycloalkyl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is C₃₋₈cycloalkyl-C₁₋₈alkyl, wherein C₃₋₈cycloalkyl is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; and, wherein, each instance of C₃₋₈cycloalkyl is optionally substituted with R₅ substituents.

In one embodiment of a compound of Formula (I), R₄ is C₃₋₁₄cycloalkyl-amino, wherein C₃₋₁₄cycloalkyl is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; and, wherein, each instance of C₃₋₁₄cycloalkyl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is C₃₋₈cycloalkyl-amino, wherein C₃₋₈cycloalkyl is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl; and, wherein, each instance of C₃₋₈cycloalkyl is optionally substituted with R₅ substituents.

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In one embodiment of a compound of Formula (I), R₄ is aryl-C₁₋₈alkyl, aryl-C₁₋₈alkoxy-carbonyl or aryl-sulfonyloxy-C₁₋₈alkyl, wherein aryl is selected from phenyl; and, wherein, each instance of aryl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is aryl-C₁₋₈alkyl or aryl-C₁₋₈alkoxy-carbonyl, wherein each instance of aryl is optionally substituted with R₅ substituents.

In one embodiment of a compound of Formula (I), R₄ is heterocyclyl selected from oxetanyl, pyrrolidinyl, piperidinyl, piperazinyl, 1,3-dioxanyl or morpholinyl, wherein each instance of heterocyclyl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is heterocyclyl selected from oxetan-3-yl, pyrrolidin-1-yl, piperidin-1-yl, piperazin-1-yl, 1,3-dioxan-5-yl or morpholin-4-yl, wherein each instance of heterocyclyl is optionally substituted with R₅ substituents.

In one embodiment of a compound of Formula (I), R₄ is heterocyclyl-C₁₋₈alkyl, wherein each instance of heterocyclyl is selected from pyrrolidinyl or piperidinyl; and, wherein, each instance of heterocyclyl is optionally substituted with R₅ substituents.

In another embodiment of a compound of Formula (I), R₄ is heterocyclyl-C₁₋₈alkyl selected from pyrrolidin-1-yl-C₁₋₈alkyl or piperidin-1-yl-C₁₋₈alkyl, wherein each instance of heterocyclyl is optionally substituted with R₅ substituents.

In one embodiment of a compound of Formula (I), R₅ is selected from halogen, hydroxy, cyano, nitro, halo-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio; wherein, halogen and halo is selected from fluoro, chloro, bromo or iodo.

In another embodiment of a compound of Formula (I), R₅ is hydroxy.

In one embodiment of a compound of Formula (I), R₅ is C₁₋₈alkyl selected from methyl, ethyl, propyl, isopropyl, n-butyl or tert-butyl.

In another embodiment of a compound of Formula (I), R₅ is C₁₋₈alkyl selected from ethyl, propyl, isopropyl or tert-butyl.

In one embodiment of a compound of Formula (I), R₅ is halo-C₁₋₈alkyl selected from trihalo-methyl, dihalo-methyl, halo-methyl, trihalo-ethyl, dihalo-ethyl, halo-ethyl, trihalo-propyl, dihalo-propyl or halo-propyl; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R₅ is C₁₋₈alkoxy selected from methoxy, ethoxy, propoxy or isopropoxy.

In one embodiment of a compound of Formula (I), R₅ is halo-C₁₋₈alkoxy selected from trihalo-methoxy, dihalo-methoxy, halo-methoxy, trihalo-ethoxy, dihalo-ethoxy, halo-ethoxy, trihalo-propoxy, dihalo-propoxy or halo-propoxy; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R₂ is aryl selected from phenyl optionally substituted with R₆ and R₇ substituents.

In one embodiment of a compound of Formula (I), R₂ is aryl-amino, wherein aryl is selected from phenyl; and, wherein, each instance of aryl is optionally substituted with R₆ and R₇ substituents.

In another embodiment of a compound of Formula (I), R₂ is aryl-amino selected from phenyl-amino; wherein, each instance of aryl is optionally substituted with R₆ and R₇ substituents.

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In one embodiment of a compound of Formula (I), R₂ is aryl-amino-carbonyl, wherein aryl is selected from phenyl; and, wherein, each instance of aryl is optionally substituted with R₆ and R₇ substituents.

In another embodiment of a compound of Formula (I), R₂ is aryl-amino-carbonyl selected from phenyl-amino-carbonyl; wherein, each instance of aryl is optionally substituted with R₆ and R₇ substituents.

In one embodiment of a compound of Formula (I), R₂ is heterocyclyl selected from 1,2,3,6-tetrahydropyridinyl, 1,3-benzodioxolyl or 2,3-dihydro-1,4-benzodioxinyl; wherein, each instance of heterocyclyl is optionally substituted with R₆ and R₇ substituents.

In another embodiment of a compound of Formula (I), R₂ is heterocyclyl selected from 1,2,3,6-tetrahydropyridin-4-yl, 1,3-benzodioxol-5-yl or 2,3-dihydro-1,4-benzodioxin-6-yl; wherein, each instance of heterocyclyl is optionally substituted with R₆ and R₇ substituents.

In one embodiment of a compound of Formula (I), R₂ is heteroaryl selected from thienyl, 1H-pyrazolyl, 1H-imidazolyl, 1,3-thiazolyl, 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl, pyridinyl, pyrimidinyl, 1H-indolyl, 2H-indolyl, 1H-indazolyl, 2H-indazolyl, indoliziny, benzofuranyl, benzothienyl, 1H-benzimidazolyl, 1,3-benzothiazolyl, 1,3-benzoxazolyl, 9H-purinyl, furo[3,2-b]pyridinyl, furo[3,2-c]pyridinyl, furo[2,3-c]pyridinyl, thieno[3,2-c]pyridinyl, thieno[2,3-c]pyrimidinyl, 1H-pyrrolo[2,3-b]pyridinyl, 1H-pyrrolo[2,3-c]pyridinyl, pyrrolo[1,2-c]pyrimidinyl, pyrrolo[1,2-c]pyrazinyl, pyrrolo[1,2-b]pyridazinyl, pyrazolo[1,5-c]pyridinyl, pyrazolo[1,5-c]pyrazinyl, imidazo[1,2-c]pyridinyl, imidazo[1,2-c]pyrimidinyl, imidazo[1,2-c]pyrimidinyl, imidazo[1,2-b]pyridazinyl, imidazo[1,2-c]pyrazinyl, imidazo[2,1-b][1,3]thiazolyl, imidazo[2,1-b][1,3,4]thiadiazolyl, [1,3]oxazolo[4,5-b]pyridinyl or quinoxalinyl; wherein, each instance of heteroaryl is optionally substituted with R₆ and R₇ substituents.

In another embodiment of a compound of Formula (I), R₂ is heteroaryl selected from thien-2-yl, thien-3-yl, 1H-pyrazol-3-yl, 1H-pyrazol-4-yl, 1H-pyrazol-5-yl, 1H-imidazol-1-yl, 1H-imidazol-4-yl, 1,3-thiazol-2-yl, 1,2,4-oxadiazol-3-yl, 1,3,4-oxadiazol-2-yl, pyridin-2-yl, pyridin-3-yl, pyridin-4-yl, pyrimidin-4-yl, 1H-indol-3-yl, 1H-indol-4-yl, 1H-indol-5-yl, 1H-indol-6-yl, 1H-indazol-5-yl, 2H-indazol-5-yl, indolizin-2-yl, benzofuran-2-yl, benzofuran-5-yl, benzothien-2-yl, benzothien-3-yl, 1H-benzimidazol-2-yl, 1H-benzimidazol-6-yl, 1,3-benzoxazol-2-yl, 1,3-benzoxazol-5-yl, 1,3-benzoxazol-6-yl, 1,3-benzothiazol-2-yl, 1,3-benzothiazol-5-yl, 1,3-benzothiazol-6-yl, 9H-purin-8-yl, furo[3,2-b]pyridin-2-yl, furo[3,2-c]pyridin-2-yl, furo[2,3-c]pyridin-2-yl, thieno[3,2-c]pyridin-2-yl, thieno[2,3-c]pyrimidin-6-yl, 1H-pyrrolo[2,3-b]pyridin-5-yl, 1H-pyrrolo[2,3-c]pyridin-4-yl, pyrrolo[1,2-c]pyrimidin-7-yl, pyrrolo[1,2-c]pyrazin-7-yl, pyrrolo[1,2-b]pyridazin-2-yl, pyrazolo[1,5-c]pyridin-2-yl, pyrazolo[1,5-c]pyrazin-2-yl, imidazo[1,2-c]pyridin-2-yl, imidazo[1,2-c]pyridin-6-yl, imidazo[1,2-c]pyrimidin-2-yl, imidazo[1,2-c]pyrimidin-6-yl, imidazo[1,2-c]pyrimidin-2-yl, imidazo[1,2-b]pyridazin-2-yl, imidazo[1,2-c]pyrazin-2-yl, imidazo[2,1-b][1,3]thiazol-6-yl, imidazo[2,1-b][1,3,4]thiadiazol-6-yl, [1,3]oxazolo[4,5-b]pyridin-2-yl or quinoxalin-2-yl; wherein, each instance of heteroaryl is optionally substituted with R₆ and R₇ substituents.

In another embodiment of a compound of Formula (I), R₂ is substituted heteroaryl selected from 4-methylthien-2-yl, 1-methyl-1H-pyrazol-3-yl, 4-methyl-1H-pyrazol-3-yl, 1-phenyl-1H-pyrazol-3-yl, 1-phenyl-1H-imidazol-4-yl, 2-methyl-1-(pyridin-2-yl)-1H-imidazol-4-yl, 4-methyl-1,3-thiazol-2-yl, 4-(trifluoromethyl)-1,3-thiazol-2-yl, 4-phenyl-

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1,3-thiazol-2-yl, 5-phenyl-1,2,4-oxadiazol-3-yl, 3-fluoropyridin-4-yl, 6-fluoropyridin-2-yl, 2-chloropyridin-4-yl, 4-chloropyridin-3-yl, 5-chloropyridin-2-yl, 6-methylpyridin-3-yl, 2-(trifluoromethyl)pyridin-3-yl, 4-(trifluoromethyl)pyridin-2-yl, 6-(trifluoromethyl)pyridin-2-yl, 2-methoxypyridin-4-yl, 4-methoxypyridin-3-yl, 6-methoxypyridin-2-yl, 2-ethoxypyridin-3-yl, 6-ethoxypyridin-2-yl, 6-(propan-2-yloxy)pyridin-2-yl, 6-(dimethylamino)pyridin-3-yl, 6-(methylsulfanyl)pyridin-2-yl, 6-(cyclobutyl)pyridin-2-yl, 6-(pyrrolidin-1-yl)pyridin-2-yl, 2-methylpyrimidin-4-yl, 2-(propan-2-yl)pyrimidin-4-yl, 2-cyclopropylpyrimidin-4-yl, 1-methyl-1H-indol-3-yl, 2-methyl-2H-indazol-5-yl, 2-methyl-1-benzofuran-5-yl, 1-methyl-1H-benzimidazol-2-yl, 4-methyl-1H-benzimidazol-2-yl 5-fluoro-1H-benzimidazol-2-yl, 4-fluoro-1,3-benzoxazol-2-yl, 5-fluoro-1,3-benzoxazol-2-yl, 7-chloro-1,3-benzoxazol-2-yl, 4-iodo-1,3-benzoxazol-2-yl, 2-methyl-1,3-benzoxazol-6-yl, 4-methyl-1,3-benzoxazol-2-yl, 4-(trifluoromethyl)-1,3-benzoxazol-2-yl, 7-(trifluoromethyl)-1,3-benzoxazol-2-yl, 2-methyl-1,3-benzothiazol-2-yl, 2-methyl-1,3-benzothiazol-5-yl, 2-methyl-1,3-benzothiazol-6-yl, 4-chloro-1,3-benzothiazol-2-yl, 7-chloro-1,3-benzothiazol-2-yl, 4-(trifluoromethyl)-1,3-benzothiazol-2-yl, 5-methylfuro[3,2-b]pyridin-2-yl, 4,6-dimethylfuro[3,2-c]pyridin-2-yl, 5,7-dimethylfuro[2,3-c]pyridin-2-yl, 4,6-dimethylthieno[3,2-c]pyridin-2-yl, 2,4-dimethylthieno[2,3-d]pyrimidin-6-yl, 1-methylpyrrolo[1,2-a]pyrazin-7-yl, 3-methylpyrrolo[1,2-a]pyrazin-7-yl, 1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl, 2-methylpyrrolo[1,2-b]pyridazin-2-yl, 4,6-dimethylpyrazolo[1,5-a]pyrazin-2-yl, 5-methylpyrazolo[1,5-a]pyridin-2-yl, 4,6-dimethylpyrazolo[1,5-a]pyrazin-2-yl, 2-chloroimidazo[2,1-b][1,3]thiazol-6-yl, 2-methylimidazo[2,1-b][1,3]thiazol-6-yl, 3-methylimidazo[2,1-b][1,3]thiazol-6-yl, 2-ethylimidazo[2,1-b][1,3]thiazol-6-yl, 2-methylimidazo[2,1-b][1,3,4]thiadiazol-6-yl, 6-cyanoimidazo[1,2-a]pyridin-2-yl (also referred to as 2-imidazo[1,2-a]pyridine-6-carbonitrile), 6-fluoroimidazo[1,2-a]pyridin-2-yl, 8-fluoroimidazo[1,2-a]pyridin-2-yl, 6,8-difluoroimidazo[1,2-a]pyridin-2-yl, 7-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl, 8-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl, 6-chloroimidazo[1,2-a]pyridin-2-yl, 7-chloroimidazo[1,2-a]pyridin-2-yl, 8-chloroimidazo[1,2-a]pyridin-2-yl, 8-bromoimidazo[1,2-a]pyridin-2-yl, 2-methylimidazo[1,2-a]pyridin-2-yl, 5-methylimidazo[1,2-a]pyridin-2-yl, 6-methylimidazo[1,2-a]pyridin-2-yl, 7-methylimidazo[1,2-a]pyridin-2-yl, 8-methylimidazo[1,2-a]pyridin-2-yl, 7-ethylimidazo[1,2-a]pyridin-2-yl, 8-ethylimidazo[1,2-a]pyridin-2-yl, 6,8-dimethylimidazo[1,2-a]pyridin-2-yl, 8-ethyl-6-methylimidazo[1,2-a]pyridin-2-yl, 7-methoxyimidazo[1,2-a]pyridin-2-yl, 8-methoxyimidazo[1,2-a]pyridin-2-yl, 6-fluoro-8-methylimidazo[1,2-a]pyridin-2-yl, 8-fluoro-6-methylimidazo[1,2-a]pyridin-2-yl, 8-chloro-6-methylimidazo[1,2-a]pyridin-2-yl, 6-methyl-8-nitroimidazo[1,2-a]pyridin-2-yl, 8-cyclopropylimidazo[1,2-a]pyridin-2-yl, 2-methylimidazo[1,2-a]pyridin-6-yl, 2-ethylimidazo[1,2-a]pyridin-6-yl, 2,3-dimethylimidazo[1,2-a]pyridin-6-yl, 2,8-dimethylimidazo[1,2-a]pyridin-6-yl, 2-(trifluoromethyl)imidazo[1,2-a]pyridin-6-yl, 8-chloro-2-methylimidazo[1,2-a]pyridin-6-yl, 8-fluoro-2-methylimidazo[1,2-a]pyridin-6-yl, 6-fluoroimidazo[1,2-a]pyrimidin-2-yl, 6-chloroimidazo[1,2-a]pyrimidin-2-yl, 7-methylimidazo[1,2-a]pyrimidin-2-yl, 2-methylimidazo[1,2-a]pyrimidin-6-yl, 2-methylimidazo[1,2-b]pyridazin-2-yl, 2-methyl-3-(1,2,3,6-tetrahydropyridin-4-yl)imidazo[1,2-b]pyridazin-6-yl, 6-methylimidazo[1,2-a]pyrazin-2-yl, 8-methylimidazo[1,2-a]pyrazin-2-yl, 6,8-dimethylimidazo[1,2-a]pyrazin-2-yl, 6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl, 6-methyl-8-

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(trifluoromethyl)imidazo[1,2-a]pyrazin-2-yl, 8-(methylsulfonyl)imidazo[1,2-a]pyrazin-2-yl, 2-methylimidazo[2,1-b][1,3]thiazol-6-yl, 3-methylimidazo[2,1-b][1,3]thiazol-6-yl or 2-methylimidazo[2,1-b][1,3,4]thiadiazol-6-yl.

In one embodiment of a compound of Formula (I), R_2 is heteroaryl-amino, wherein heteroaryl is selected from pyridinyl or pyrimidinyl; and, wherein, each instance of heteroaryl is optionally substituted with R_6 and R_7 substituents.

In another embodiment of a compound of Formula (I), R_2 is heteroaryl-amino selected from pyridin-2-yl-amino, pyridin-3-yl-amino or pyrimidin-2-yl-amino; wherein, each instance of heteroaryl is optionally substituted with R_6 and R_7 substituents.

In one embodiment of a compound of Formula (I), R_6 is selected from halogen, hydroxy, cyano, nitro, C_{1-8} alkyl, halo- C_{1-8} alkyl, hydroxy- C_{1-8} alkyl, C_{1-8} alkoxy, halo- C_{1-8} alkoxy, C_{1-8} alkoxy- C_{1-8} alkyl, $(C_{1-8}alkyl)_2$ -amino or $C_{1-8}alkyl$ -thio; wherein, halogen and halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R_6 is $C_{1-8}alkyl$ selected from methyl, ethyl, propyl, isopropyl or tert-butyl.

In another embodiment of a compound of Formula (I), R_6 is $C_{1-8}alkyl$ selected from ethyl, propyl, isopropyl or tert-butyl.

In one embodiment of a compound of Formula (I), R_6 is $C_{2-8}alkenyl$ selected from ethenyl, allyl or buta-1,3-dienyl.

In another embodiment of a compound of Formula (I), R_6 is $C_{2-8}alkenyl$ selected from ethenyl or allyl.

In one embodiment of a compound of Formula (I), R_6 is halo- $C_{1-8}alkyl$ selected from trihalo-methyl, dihalo-methyl, halo-methyl, trihalo-ethyl, dihalo-ethyl, halo-ethyl, trihalo-propyl, dihalo-propyl or halo-propyl; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R_6 is hydroxy- $C_{1-8}alkyl$ selected from hydroxy-methyl, hydroxy-ethyl, hydroxy-propyl, dihydroxy-propyl, hydroxy-butyl or dihydroxy-butyl.

In another embodiment of a compound of Formula (I), R_6 is hydroxy- $C_{1-8}alkyl$ selected from hydroxy-methyl, dihydroxy-propyl, hydroxy-butyl or dihydroxy-butyl.

In one embodiment of a compound of Formula (I), R_6 is $C_{1-8}alkoxy$ selected from methoxy, ethoxy, propoxy or isopropoxy.

In one embodiment of a compound of Formula (I), R_6 is halo- $C_{1-8}alkoxy$ selected from trihalo-methoxy, dihalo-methoxy, halo-methoxy, trihalo-ethoxy, dihalo-ethoxy, halo-ethoxy, trihalo-propoxy, dihalo-propoxy or halo-propoxy; wherein, halo is selected from fluoro, chloro, bromo or iodo.

In one embodiment of a compound of Formula (I), R_7 is $C_{3-14}cycloalkyl$, $C_{3-14}cycloalkyl$ -oxy, aryl, heterocyclyl or heteroaryl; wherein $C_{3-14}cycloalkyl$ is selected from cyclopropyl or cyclobutoxy; wherein aryl is selected from phenyl; wherein heterocyclyl is selected from oxetanyl, pyrrolidinyl or 1,2,3,6-tetrahydropyridinyl; and, wherein heteroaryl is selected from thienyl or pyridinyl.

In another embodiment of a compound of Formula (I), R_7 is $C_{3-14}cycloalkyl$ or $C_{3-14}cycloalkyl$ -oxy, wherein each instance of $C_{3-14}cycloalkyl$ is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.

In another embodiment of a compound of Formula (I), R_7 is $C_{3-8}cycloalkyl$ or $C_{3-8}cycloalkyl$ -oxy, wherein each instance of $C_{3-8}cycloalkyl$ is selected from cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl.

In one embodiment of a compound of Formula (I), R_7 is aryl selected from phenyl.

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In one embodiment of a compound of Formula (I), R_7 is heterocyclyl selected from oxetanyl, pyrrolidinyl or 1,2,3,6-tetrahydropyridinyl.

In another embodiment of a compound of Formula (I), R_7 is heterocyclyl selected from oxetan-3-yl, pyrrolidin-1-yl or 1,2,3,6-tetrahydropyridin-4-yl.

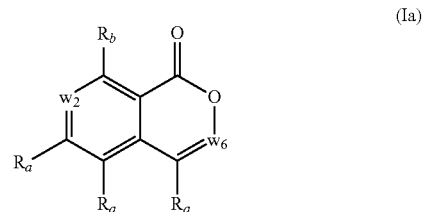
In one embodiment of a compound of Formula (I), R_7 is heteroaryl selected from thienyl or pyridinyl.

In another embodiment of a compound of Formula (I), R_7 is heteroaryl selected from pyridinyl.

In another embodiment of a compound of Formula (I), R_7 is heteroaryl selected from thien-2-yl or pyridin-2-yl.

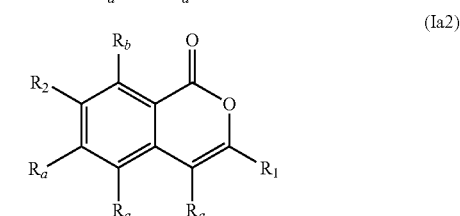
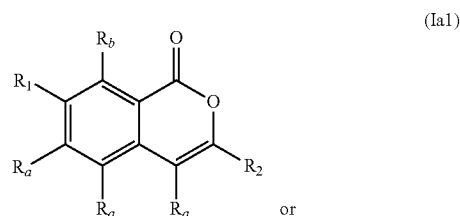
In another embodiment of a compound of Formula (I), R_7 is heteroaryl selected from pyridin-2-yl.

In one embodiment of a compound of Formula (I), the compound is selected from Formula (Ia):



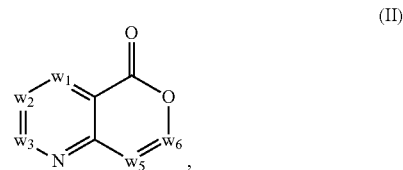
or a form thereof.

In another embodiment of a compound of Formula (Ia), the compound is selected from Formula (Ia1) or Formula (Ia2):



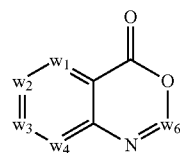
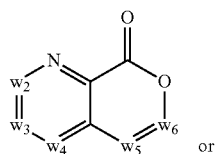
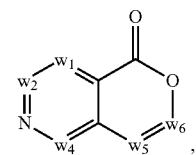
or a form thereof.

In one embodiment of a compound of Formula (I), the compound is selected from Formula (II), Formula (III), Formula (IV) or Formula (V):



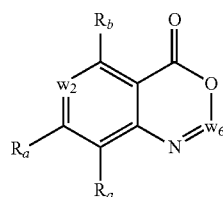
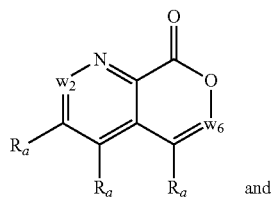
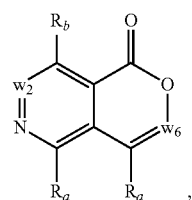
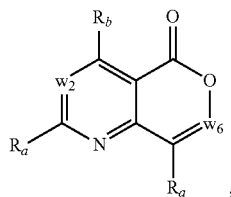
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or a form thereof.

In another embodiment of a compound of Formula (II), Formula (III), Formula (IV) and Formula (V), the compound is selected from Formula (IIa), Formula (IIIa), Formula (IVa) and Formula (Va), respectively:



or a form thereof.

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In another embodiment of a compound of Formula (IIa), the compound is selected from Formula (IIa1) or Formula (IIa2):

(III)

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(IV)

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(V)

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or a form thereof.

In another embodiment of a compound of Formula (IIIa), the compound is selected from Formula (IIIa1) or Formula (IIIa2):

(IIa)

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(IIIa)

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(IVa)

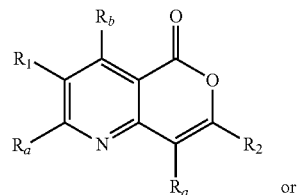
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(Va)

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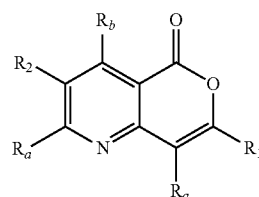
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(IIa1)

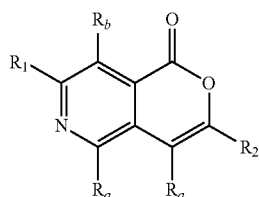


or

(IIa2)

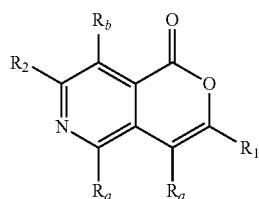


(IIIa1)



or

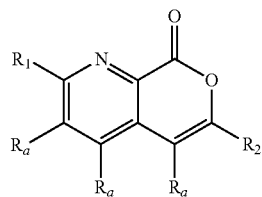
(IIIa2)



or a form thereof.

In another embodiment of a compound of Formula (IVa), the compound is selected from Formula (IVa1) or Formula (IVa2):

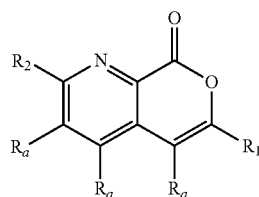
(IVa1)



or

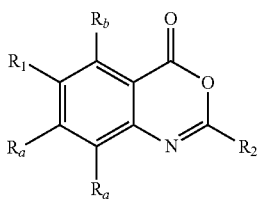
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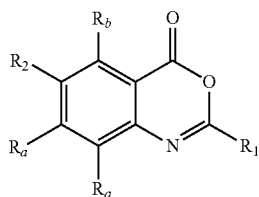


or a form thereof.

In another embodiment of a compound of Formula (Va),
the compound is selected from Formula (Va1) or Formula (Va2):

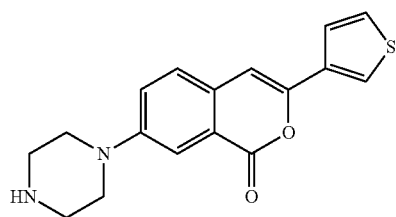
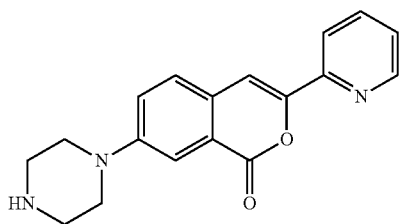


or



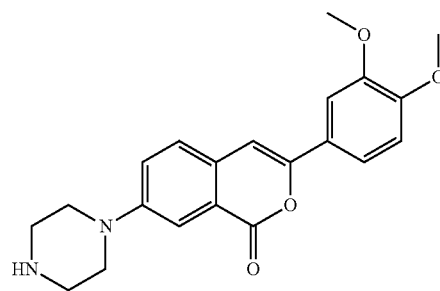
or a form thereof.

In one embodiment of a compound of Formula (I), the
compound is selected from the group consisting of:

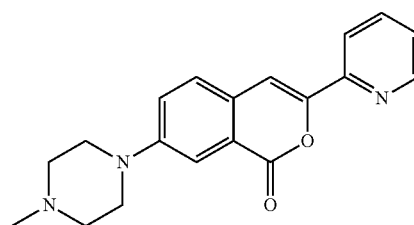
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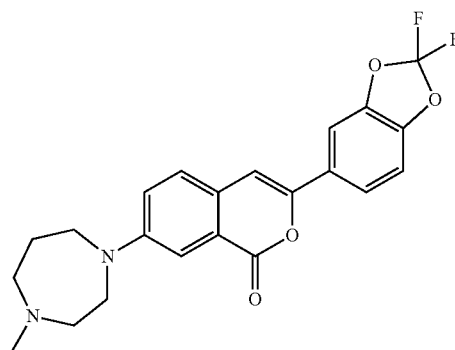
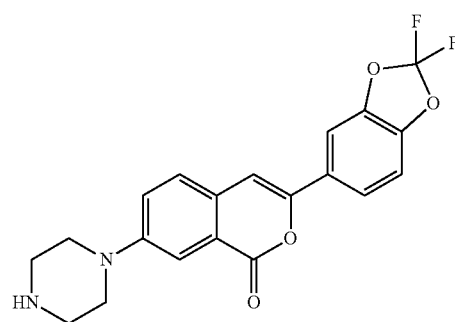
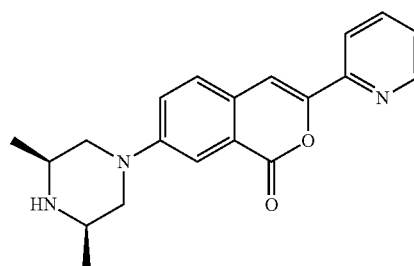
(IVa2)



(Va1)

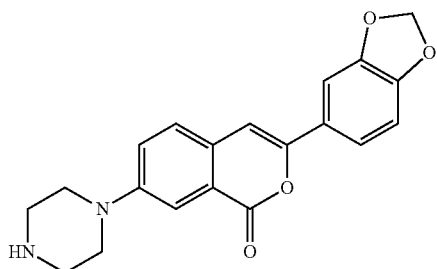
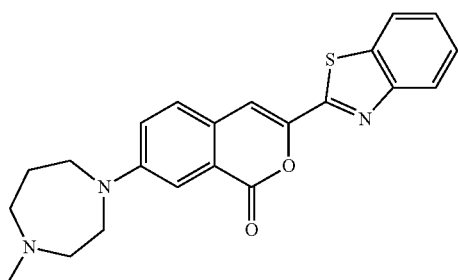
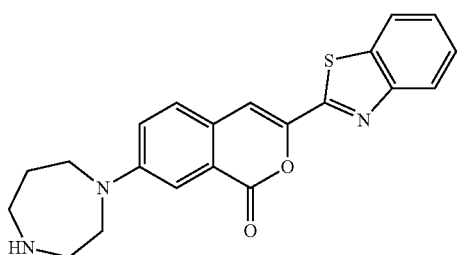
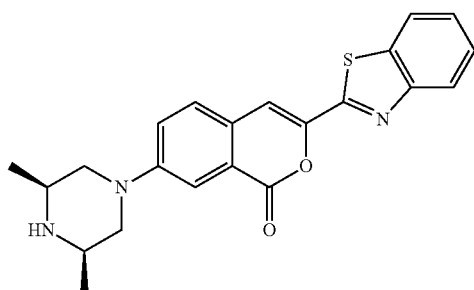
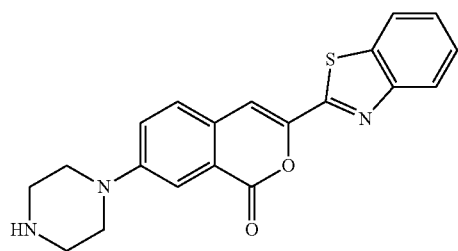


(Va2)

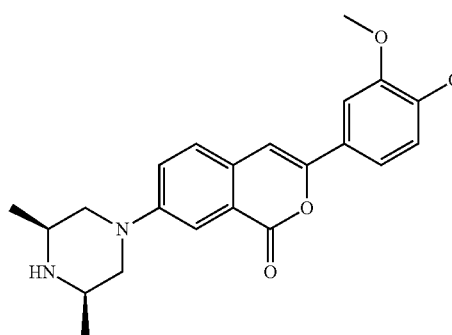
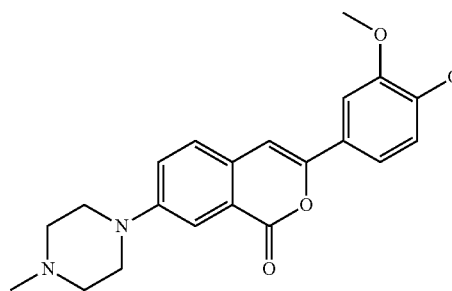
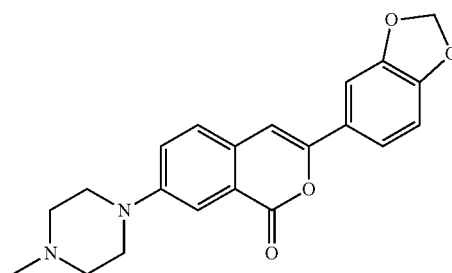
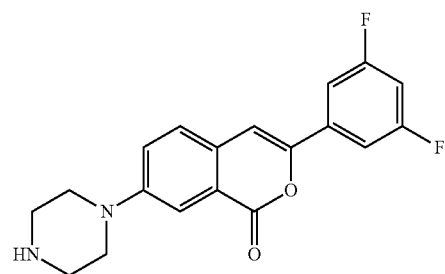
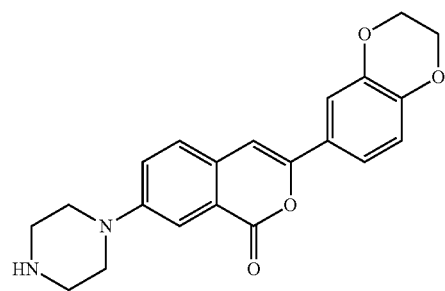


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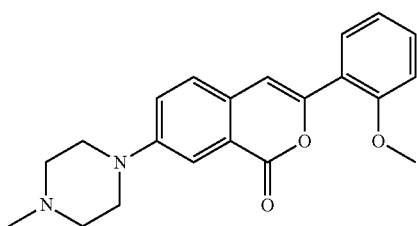
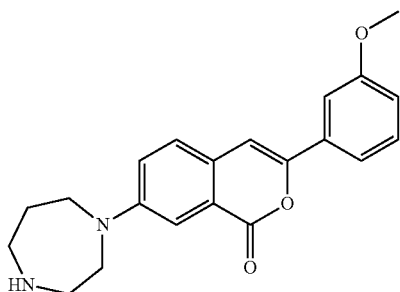
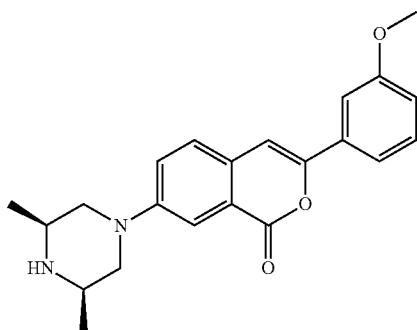
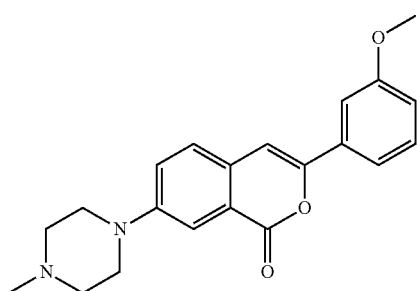
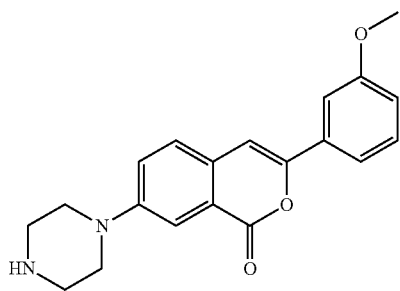
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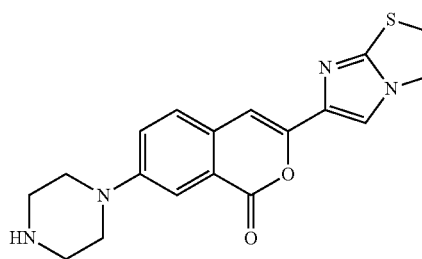
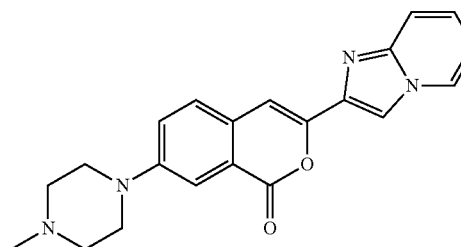
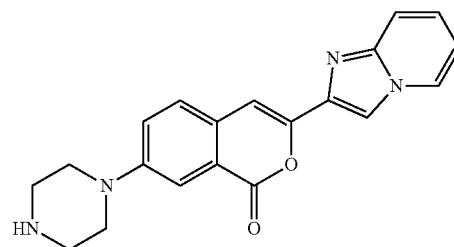
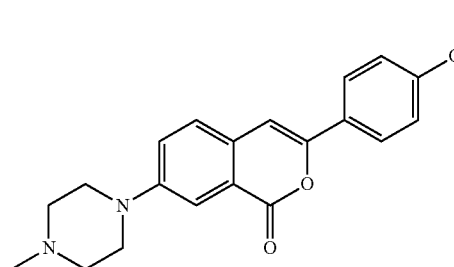
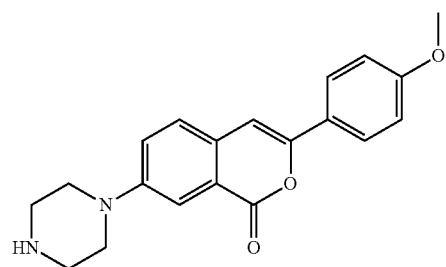
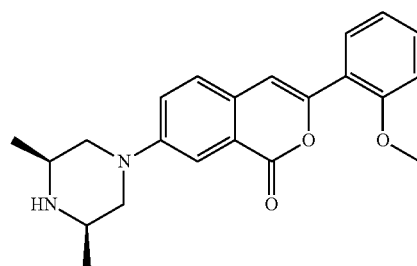
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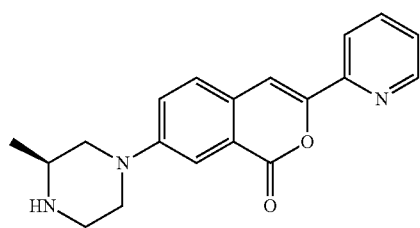
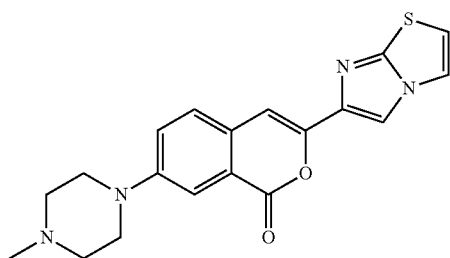
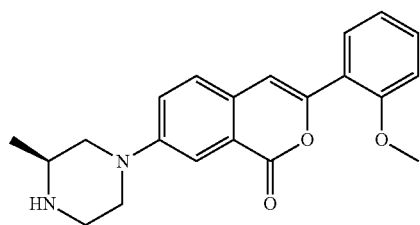
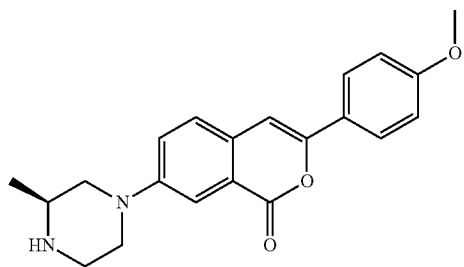
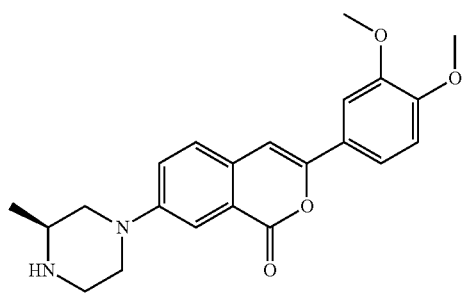
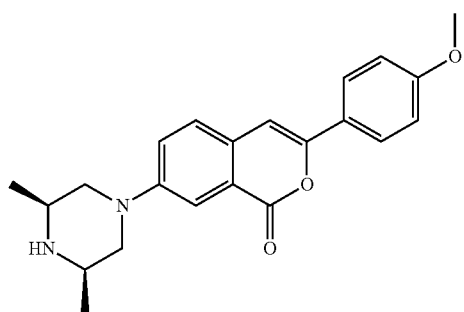
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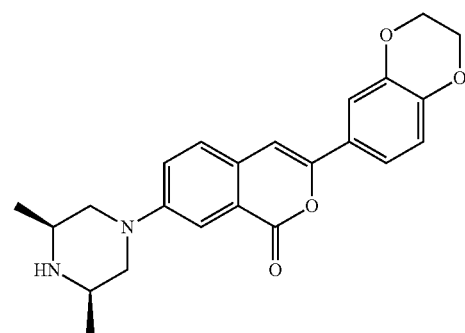
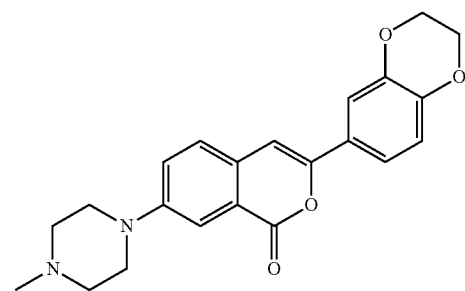
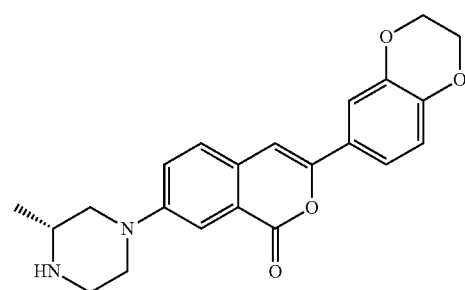
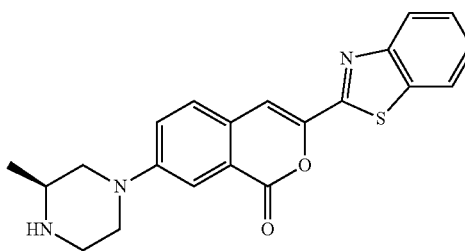
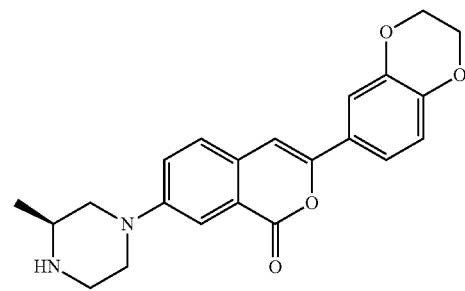
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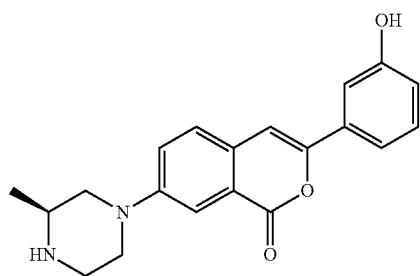
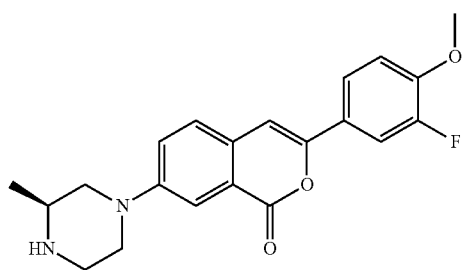
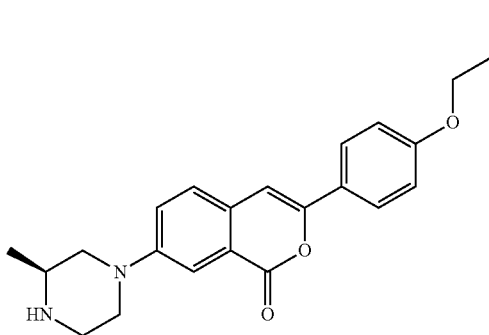
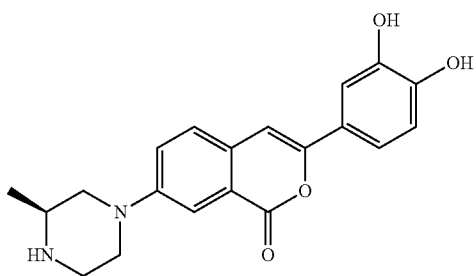
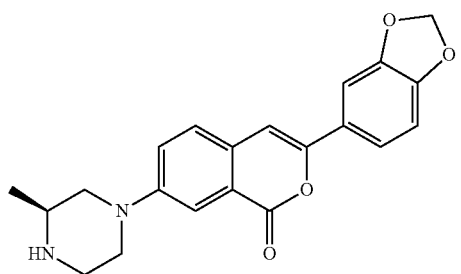
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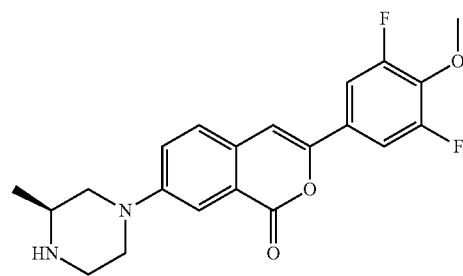
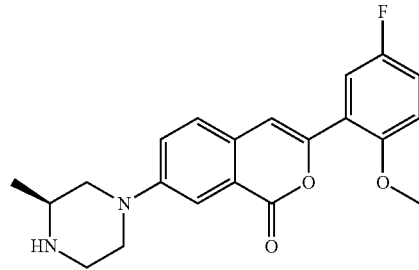
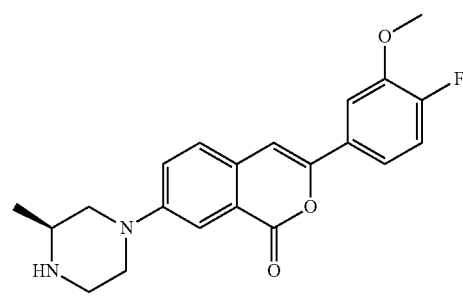
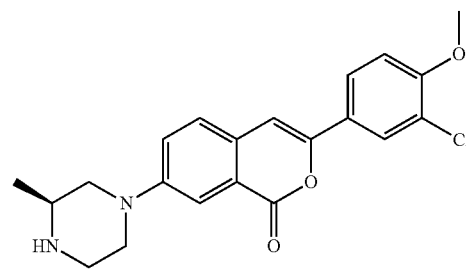
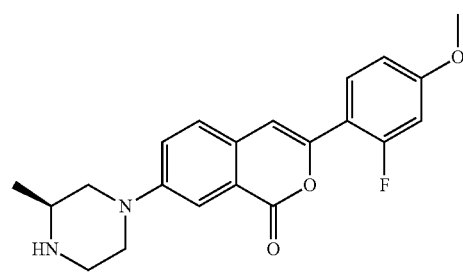


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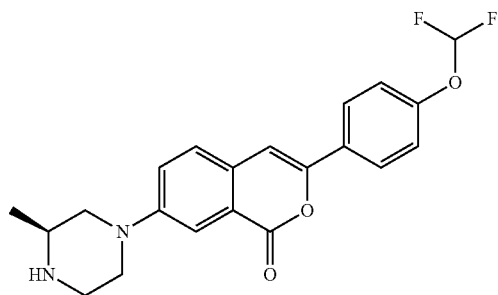
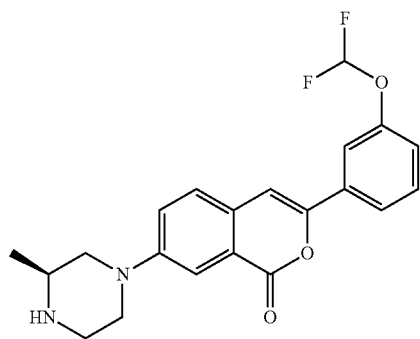
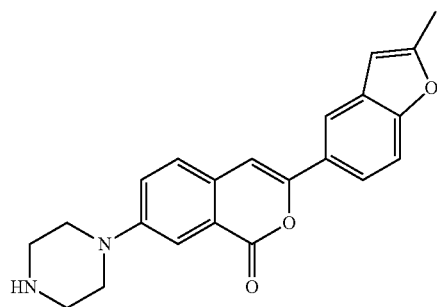
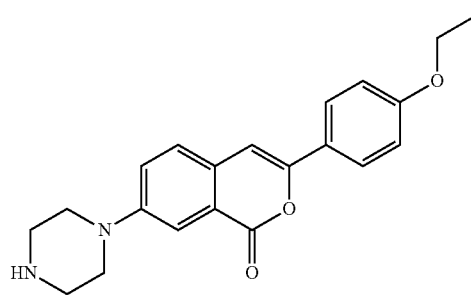
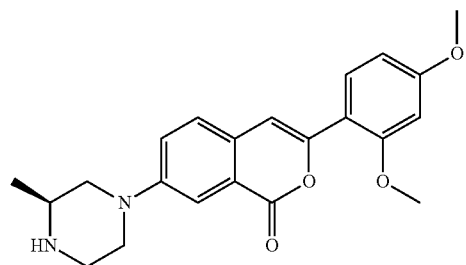
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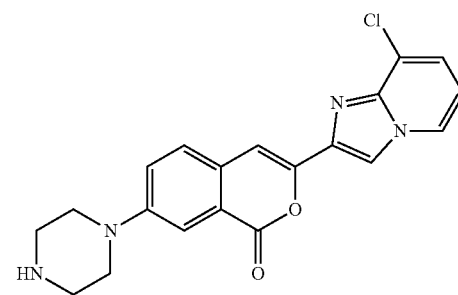
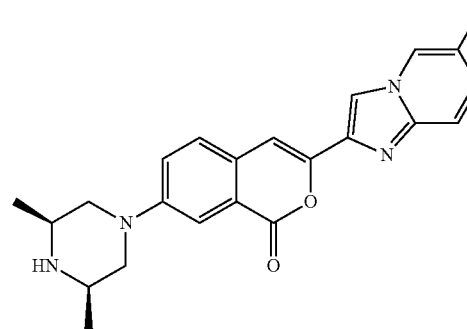
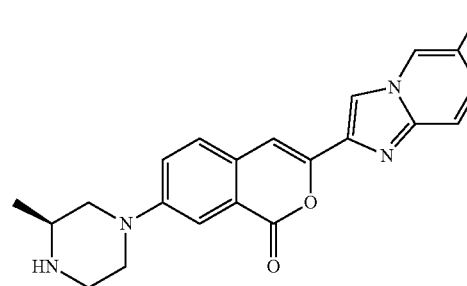
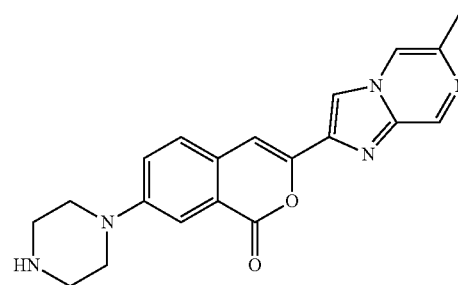
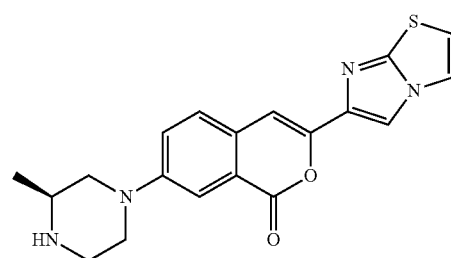


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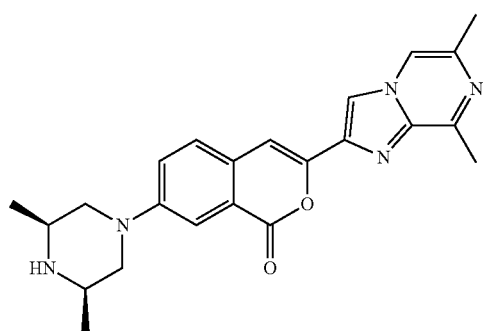
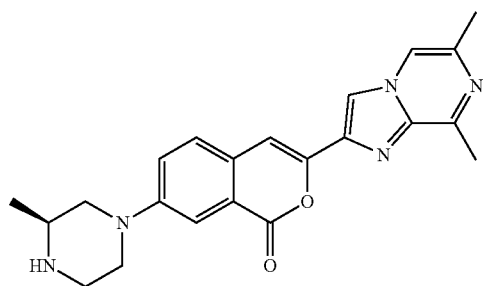
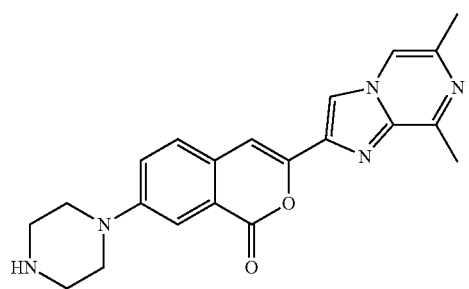
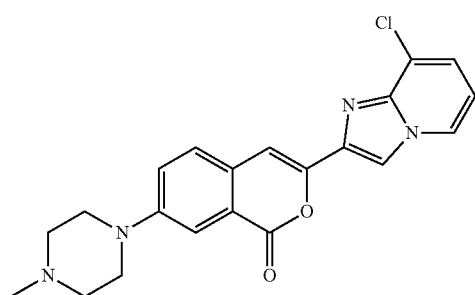
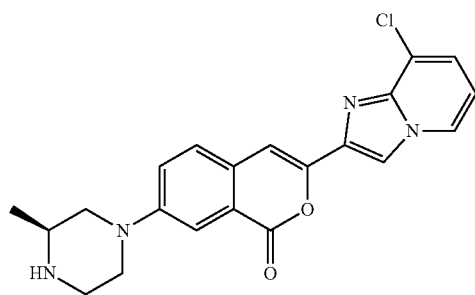
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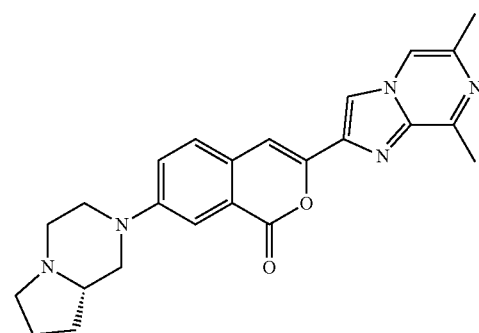
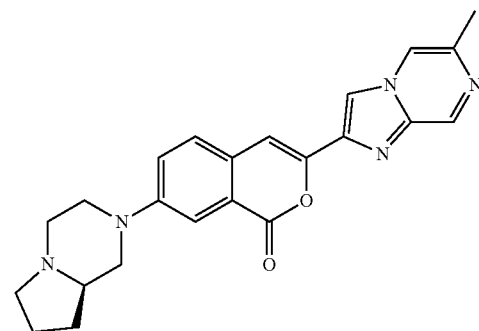
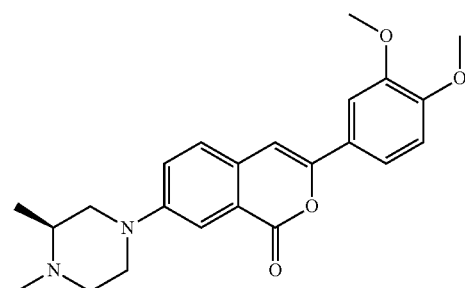
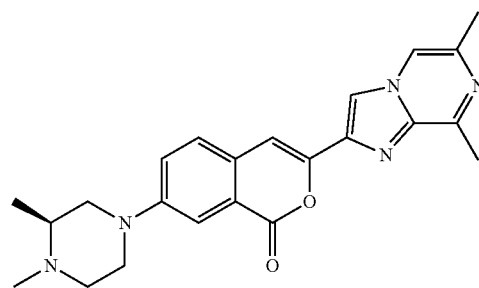
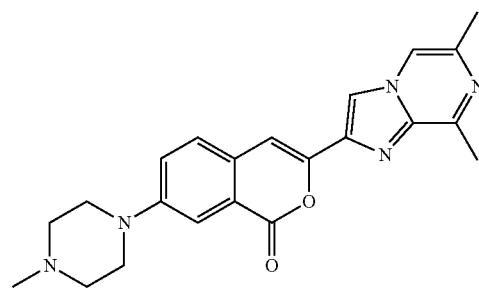
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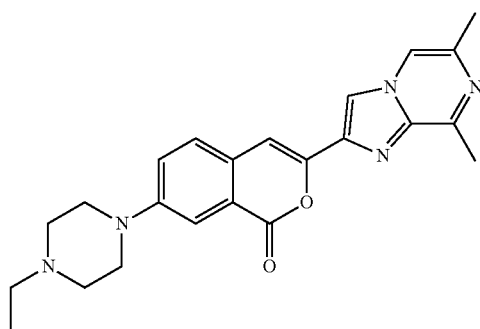
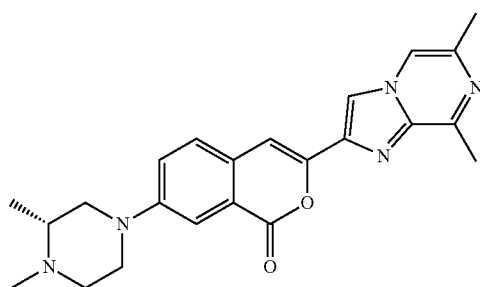
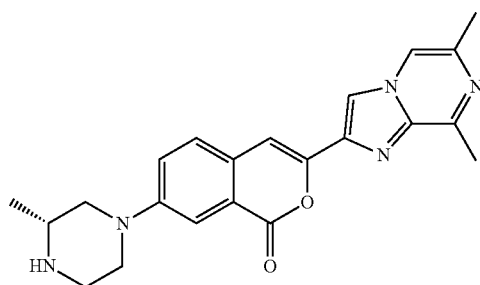
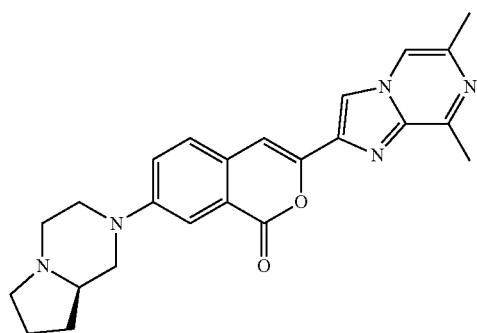
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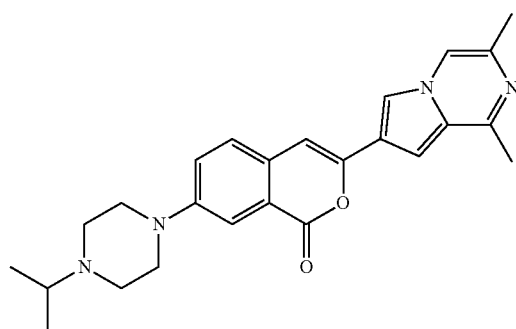
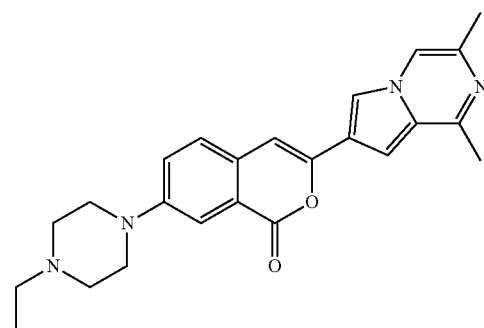
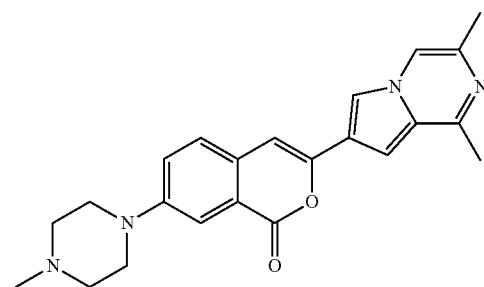
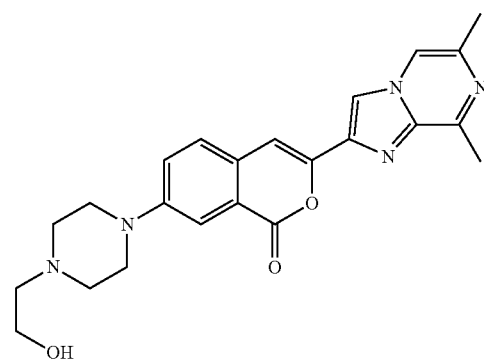
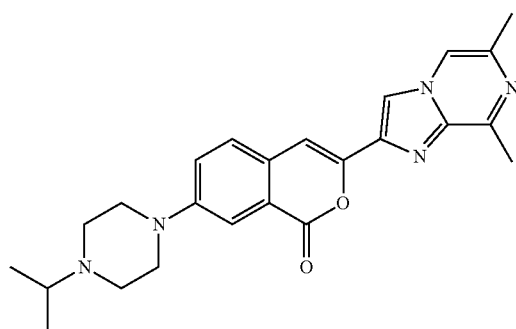


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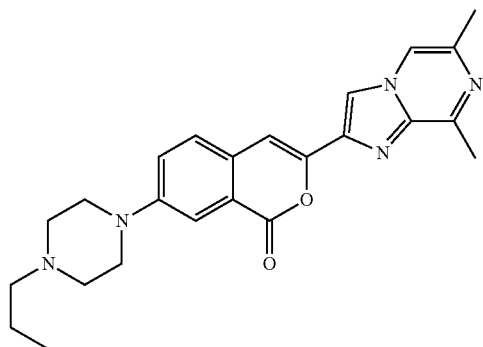
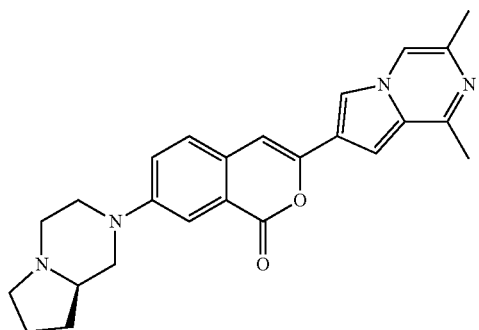
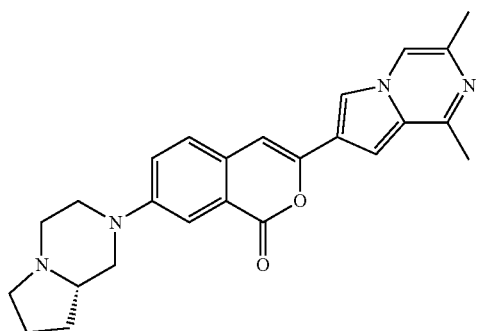
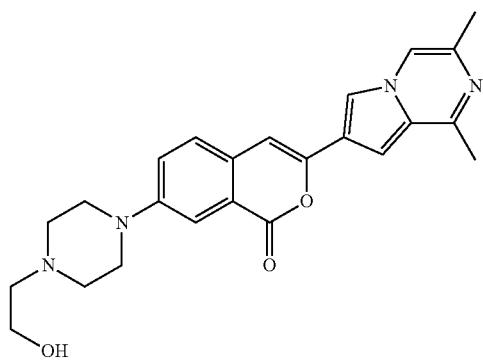
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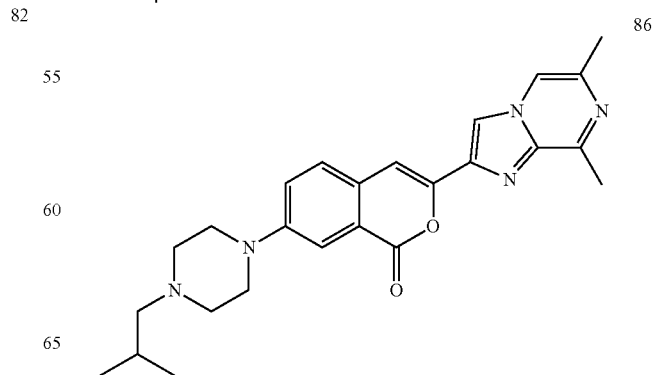
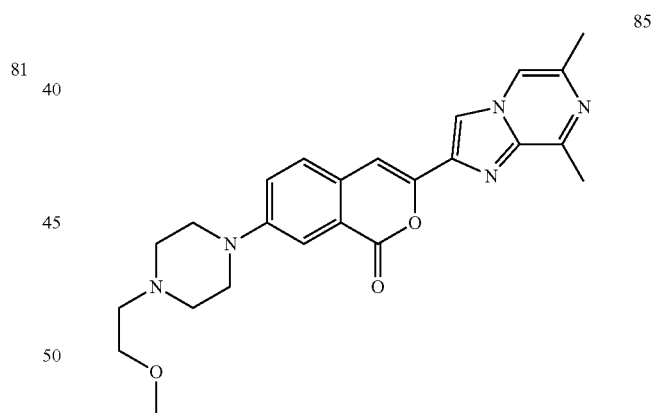
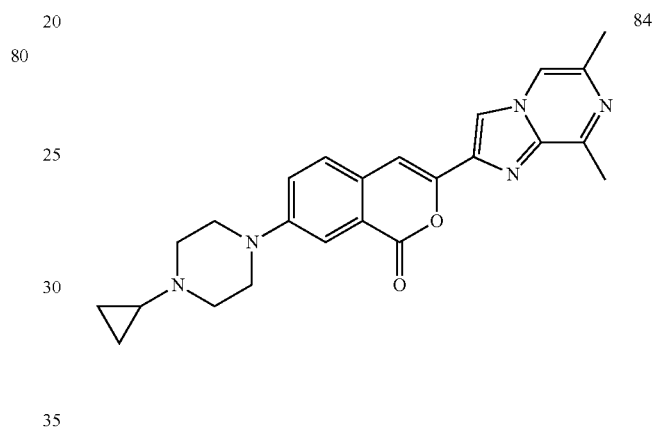
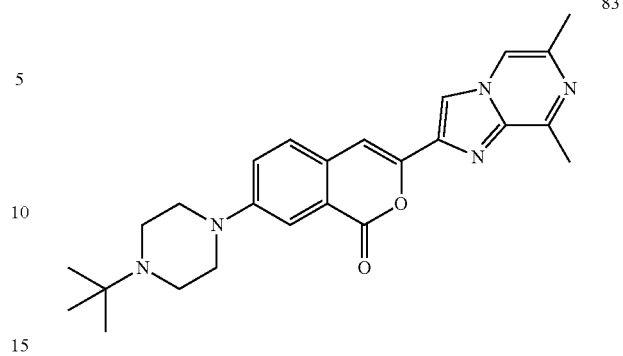


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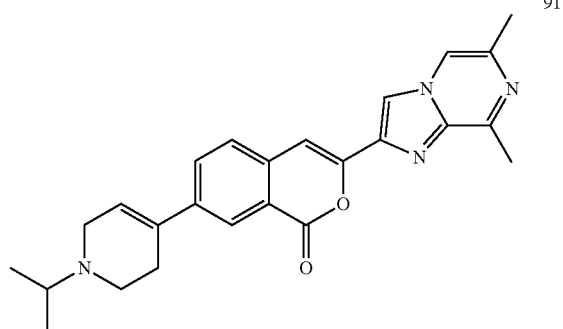
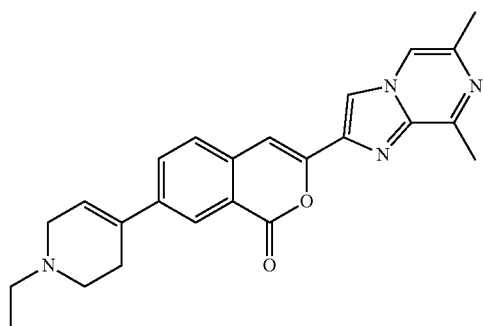
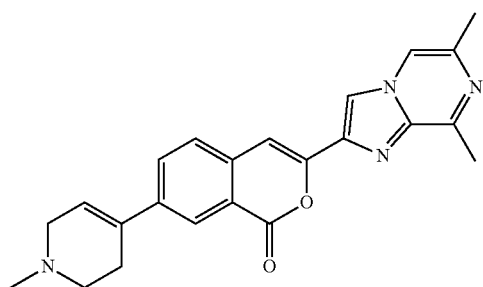
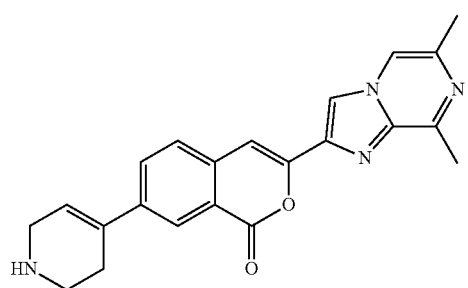
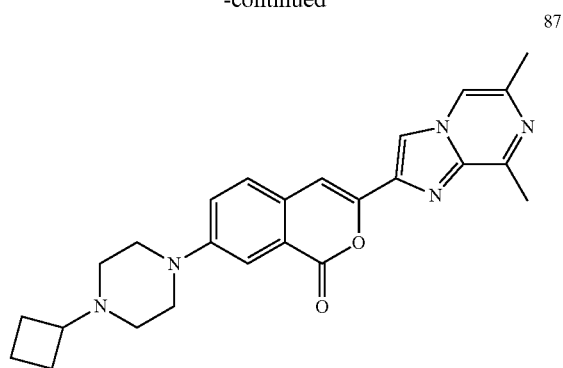
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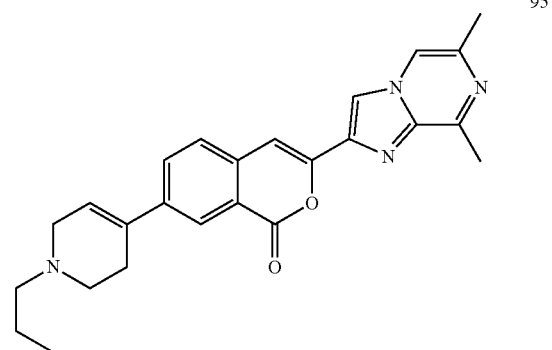
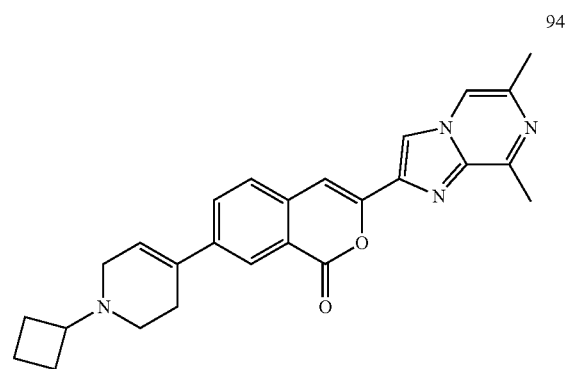
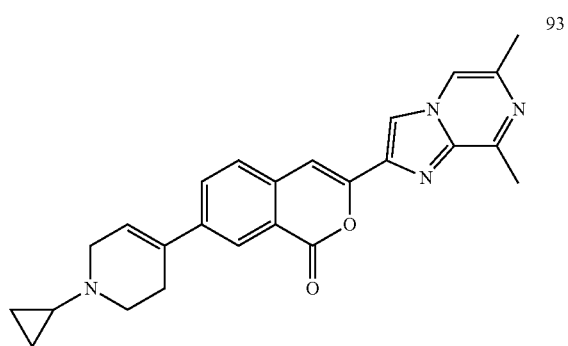
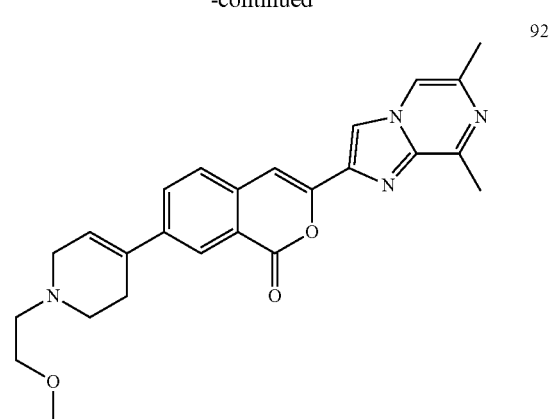


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**48**

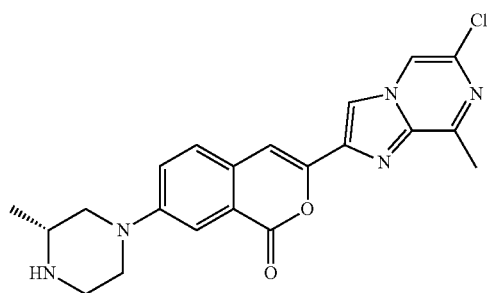
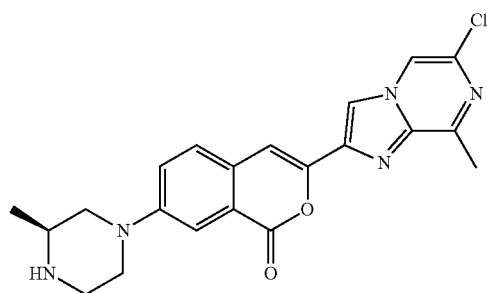
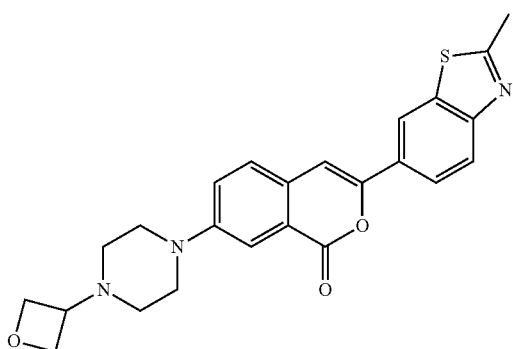
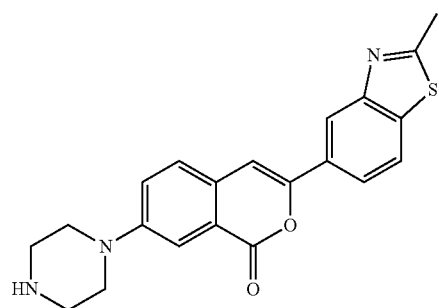
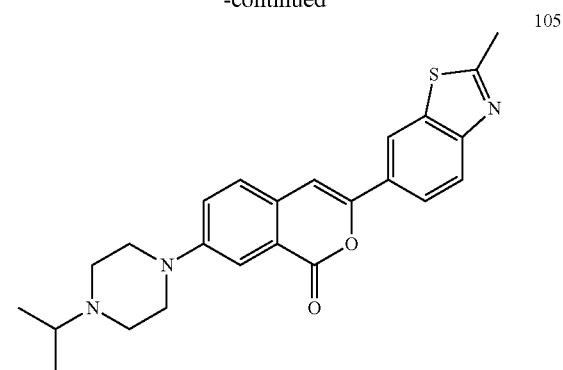
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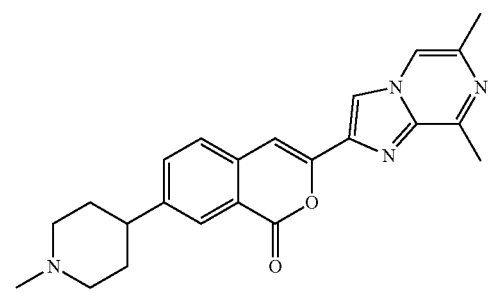
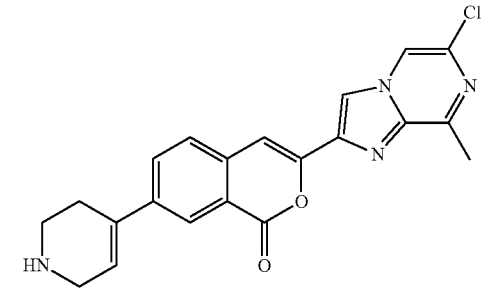
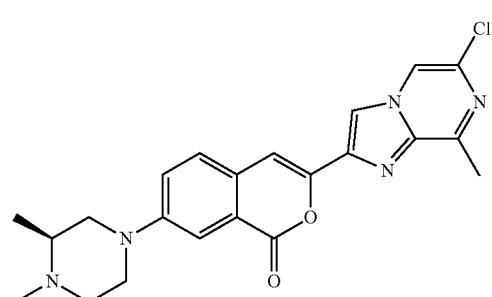
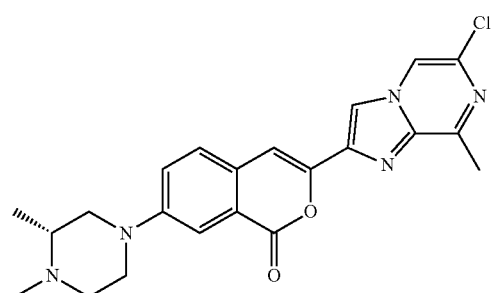
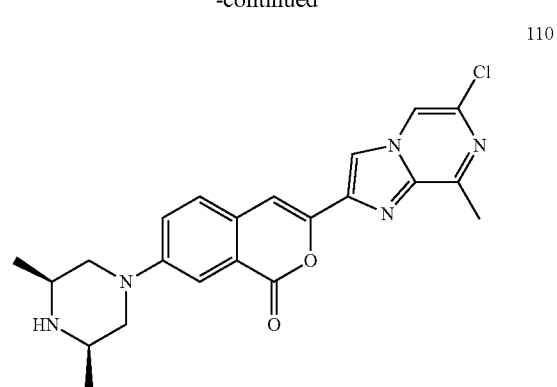


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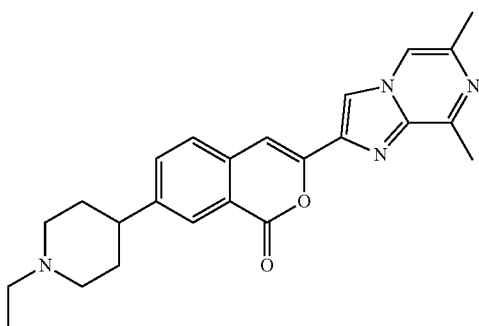
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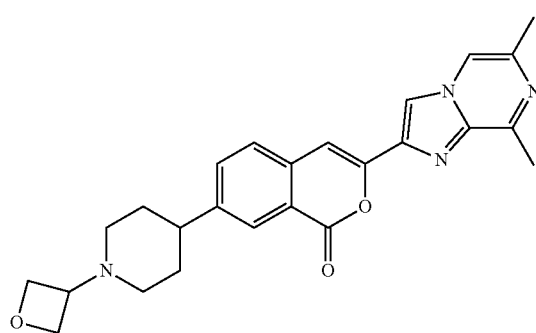
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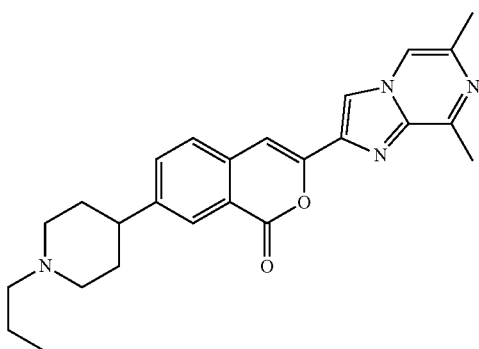
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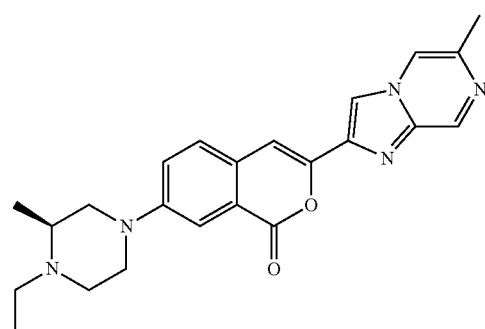


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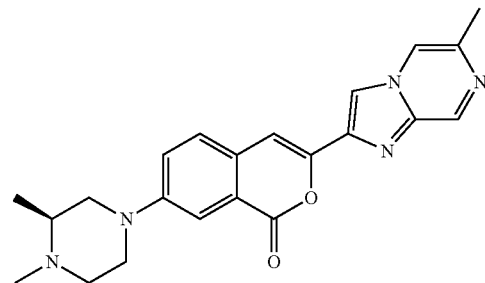
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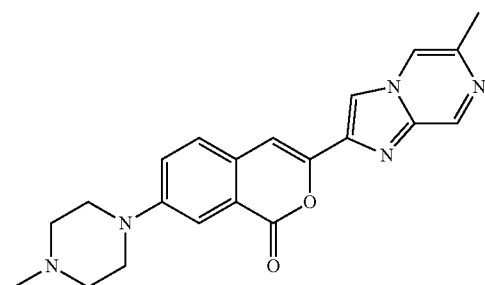


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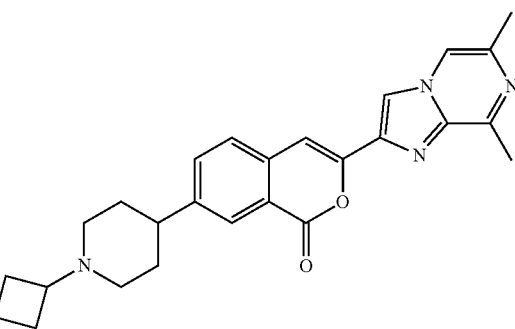
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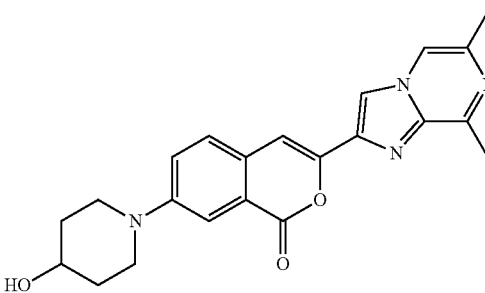


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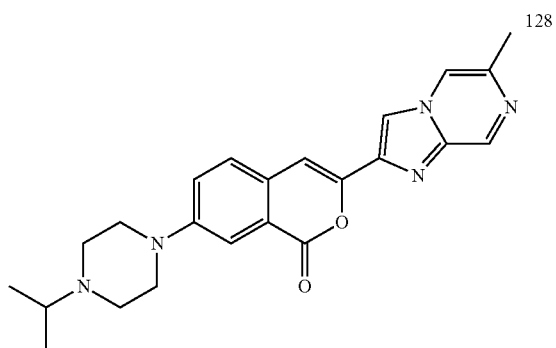
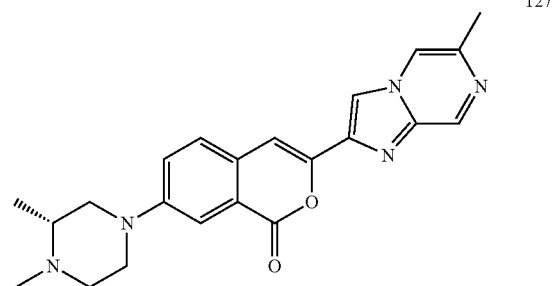
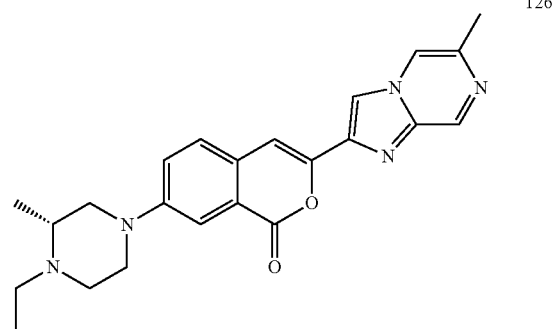
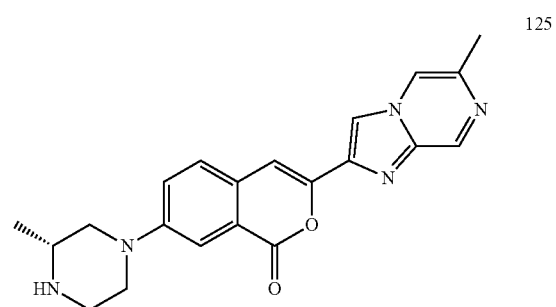
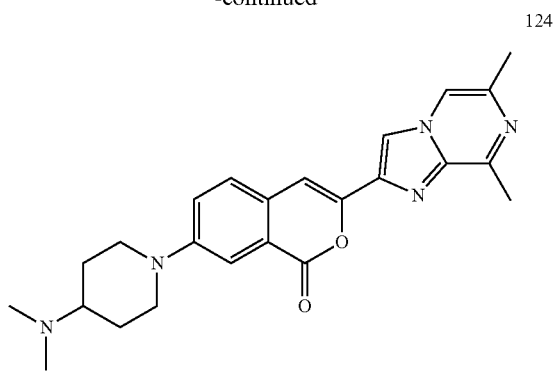
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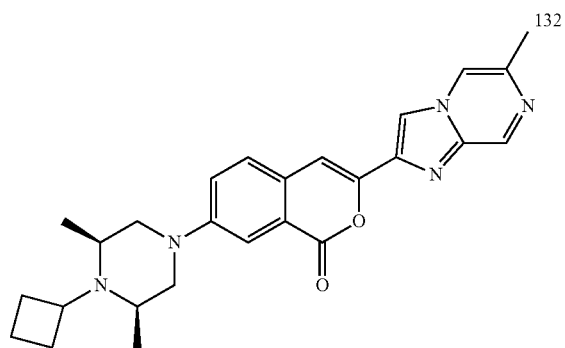
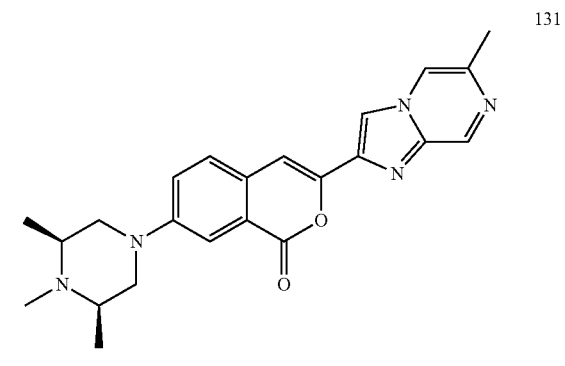
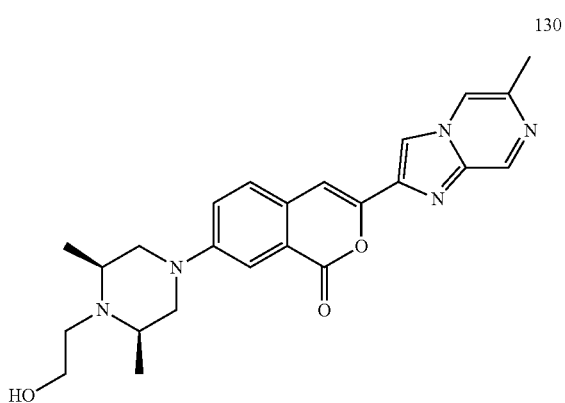
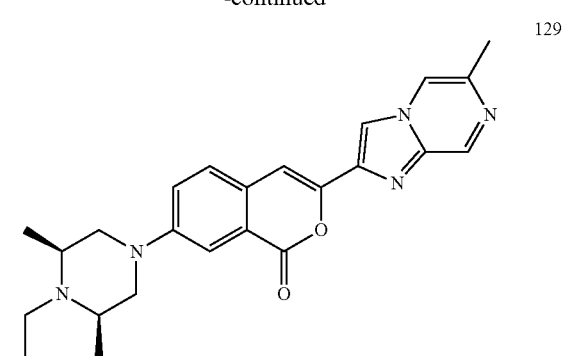
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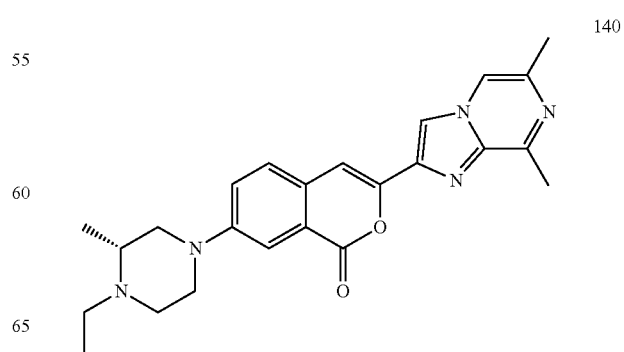
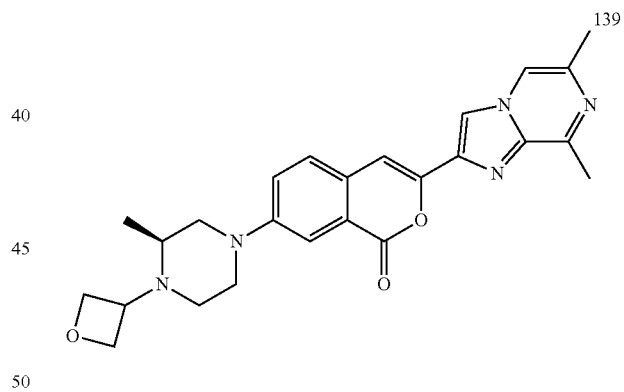
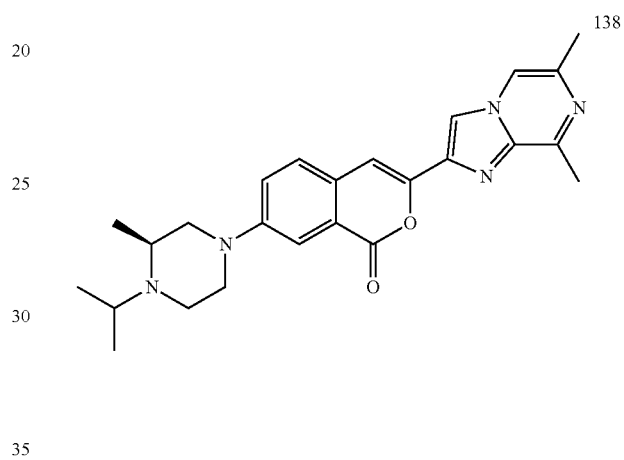
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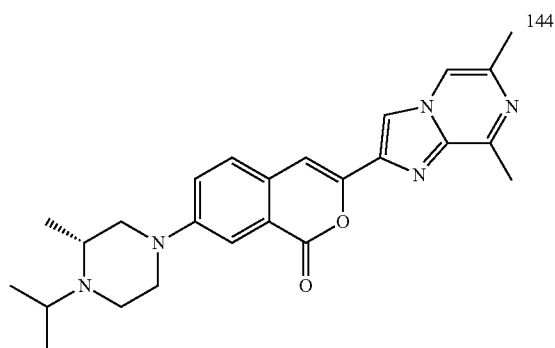
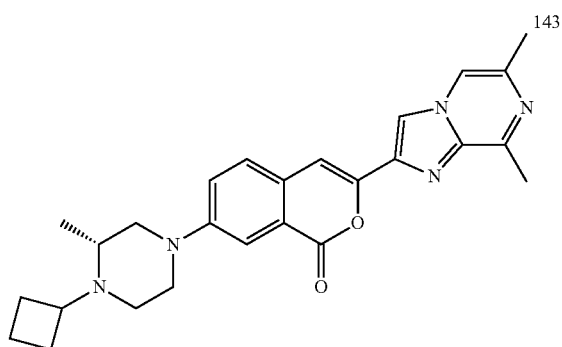
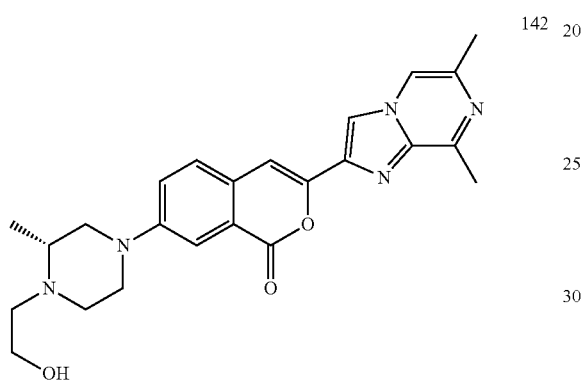
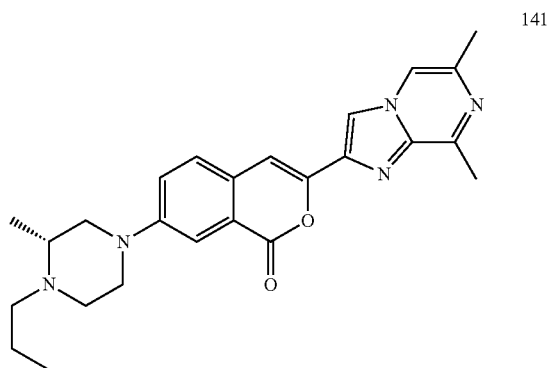
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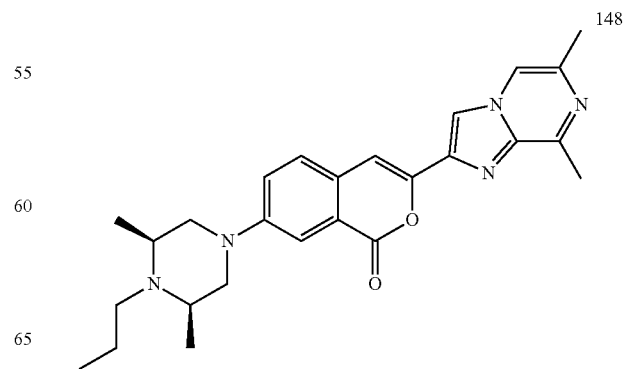
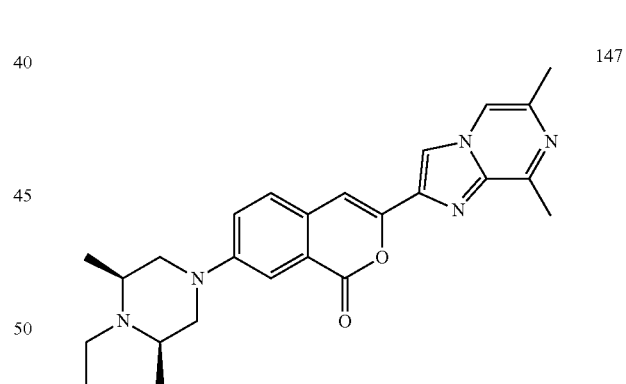
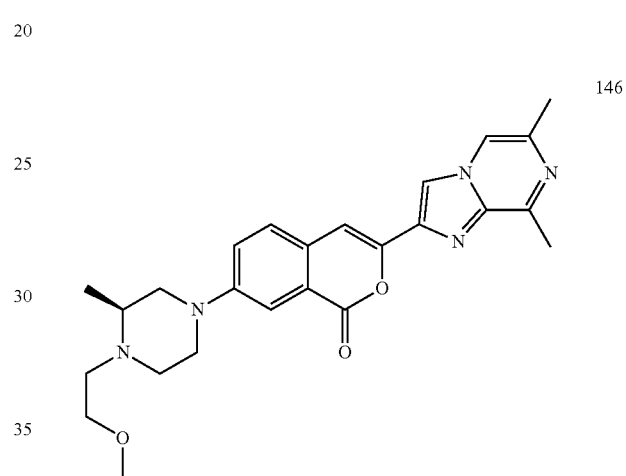
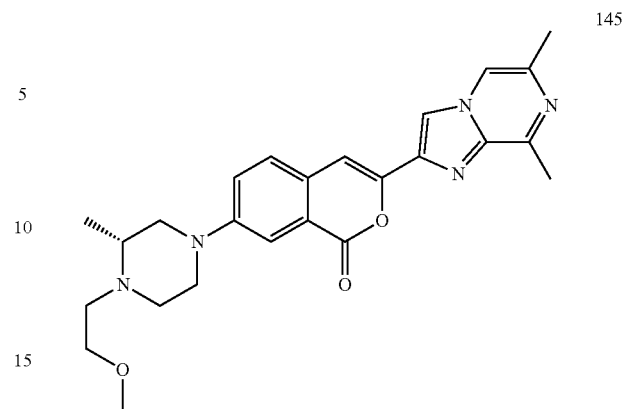


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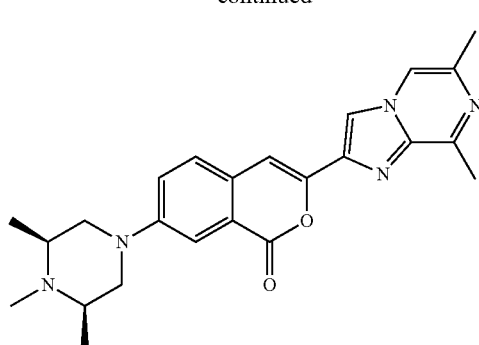
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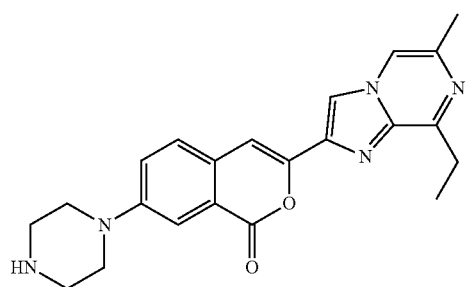


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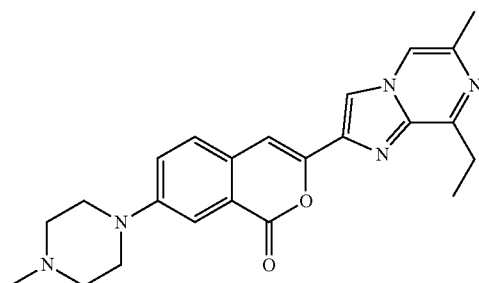


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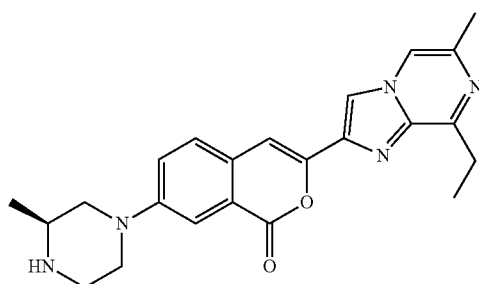
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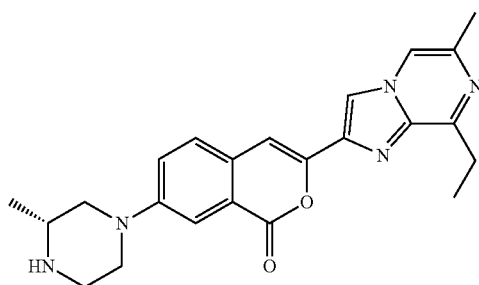
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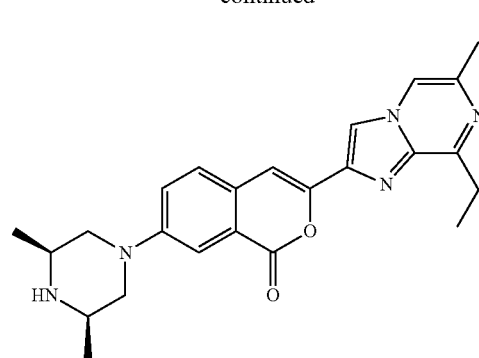
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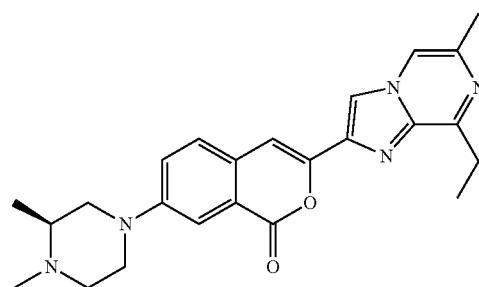
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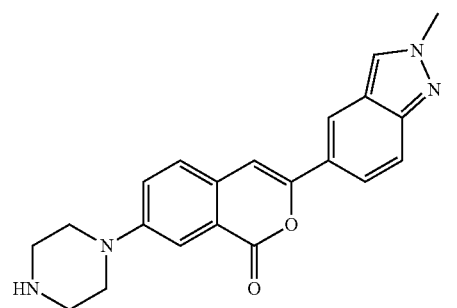
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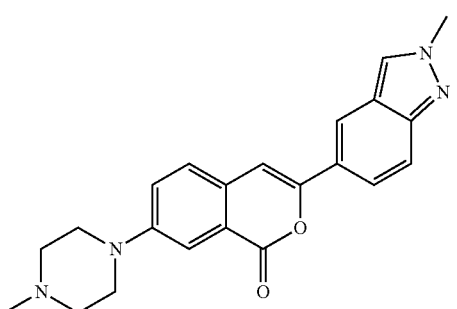
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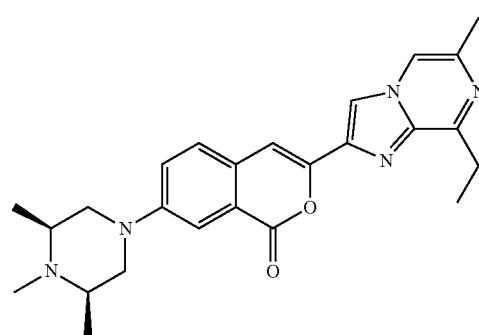
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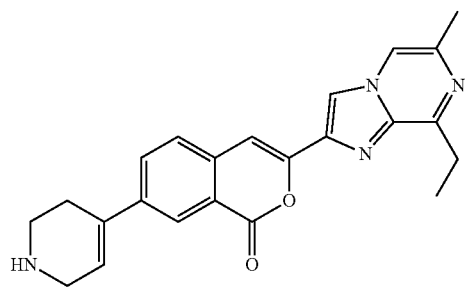
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or a form thereof.

TERMINOLOGY

The chemical terms used above and throughout the description herein, unless specifically defined otherwise, shall be understood by one of ordinary skill in the art to have the following indicated meanings.

As used herein, the term “C₁₋₈alkyl” generally refers to saturated hydrocarbon radicals having from one to eight carbon atoms in a straight or branched chain configuration, including, but not limited to, methyl, ethyl, n-propyl (also referred to as propyl or propanyl), isopropyl, n-butyl (also referred to as butyl or butanyl), isobutyl, sec-butyl, tert-butyl, n-pentyl (also referred to as pentyl or pentanyl), n-hexyl (also referred to as hexyl or hexanyl), n-heptyl (also referred to as heptyl or heptanyl), n-octyl and the like. In some embodiments, C₁₋₈alkyl includes, but is not limited to, C₁₋₆alkyl, C₁₋₄alkyl and the like. A C₁₋₈alkyl radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “C₂₋₈alkenyl” generally refers to partially unsaturated hydrocarbon radicals having from two to eight carbon atoms in a straight or branched chain configuration and one or more carbon-carbon double bonds therein, including, but not limited to, ethenyl (also referred to as vinyl), allyl, propenyl and the like. In some embodiments, C₂₋₈alkenyl includes, but is not limited to, C₂₋₆alkenyl, C₂₋₄alkenyl and the like. A C₂₋₈alkenyl radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “C₂₋₈alkynyl” generally refers to partially unsaturated hydrocarbon radicals having from two to eight carbon atoms in a straight or branched chain configuration and one or more carbon-carbon triple bonds therein, including, but not limited to, ethynyl, propynyl, butynyl and the like. In some embodiments, C₂₋₈alkynyl includes, but is not limited to, C₂₋₆alkynyl, C₂₋₄alkynyl and the like. A C₂₋₈alkynyl radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “C₁₋₈alkoxy” generally refers to saturated hydrocarbon radicals having from one to eight carbon atoms in a straight or branched chain configuration of the formula: —O—C₁₋₈alkyl, including, but not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy, n-pentoxy, n-hexoxy and the like. In some embodiments, C₁₋₈alkoxy includes, but is not limited to, C₁₋₆alkoxy, C₁₋₄alkoxy and the like. A C₁₋₈alkoxy radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “C₃₋₁₄cycloalkyl” generally refers to a saturated or partially unsaturated monocyclic, bicyclic or

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polycyclic hydrocarbon radical, including, but not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, cycloheptyl, cyclooctyl, 1H-indanyl, indenyl, tetrahydronaphthalenyl and the like. In some embodiments,

C₃₋₁₄cycloalkyl includes, but is not limited to, C₃₋₈cycloalkyl, C₅₋₈cycloalkyl, C₃₋₄₀cycloalkyl and the like. A C₃₋₁₄cycloalkyl radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “aryl” generally refers to a monocyclic, bicyclic or polycyclic aromatic carbon atom ring structure radical, including, but not limited to, phenyl, naphthyl, anthracenyl, fluorenyl, azulenyl, phenanthrenyl and the like. An aryl radical is optionally substituted with substituent species as described herein where allowed by available valences.

As used herein, the term “heteroaryl” generally refers to a monocyclic, bicyclic or polycyclic aromatic carbon atom ring structure radical in which one or more carbon atom ring members have been replaced, where allowed by structural stability, with one or more heteroatoms, such as an O, S or N atom, including, but not limited to, furanyl (also referred to as furyl), thienyl (also referred to as thiophenyl), pyrrolyl, 2H-pyrrolyl, 3H-pyrrolyl, pyrazolyl, 1H-pyrazolyl, imidazolyl, 1H-imidazolyl, isoxazolyl, isothiazolyl, oxazolyl, 1,3-thiazolyl, triazolyl (such as 1H-1,2,3-triazolyl and the like), oxadiazolyl (such as 1,2,4-oxadiazolyl, 1,3,4-oxadiazolyl and the like), thiadiazolyl, tetrazolyl (such as 1H-tetrazolyl, 2H-tetrazolyl and the like), pyridinyl (also referred to as pyridyl), pyrimidinyl, pyrazinyl, pyridazinyl, triazinyl, indolyl, 1H-indolyl, indazolyl, 1H-indazolyl, 2H-indazolyl, indolizynyl, isoindolyl, benzofuranyl, benzothiophenyl (also referred to as benzothiophenyl), benzoimidazolyl, 1H-benzoimidazolyl, 1,3-benzothiazolyl, 1,3-benzoxazolyl (also referred to as 1,3-benzooxazolyl), purinyl, 9H-purinyl, quinolynyl, isoquinolynyl, quinazolynyl, quinoxalynyl, 1,3-diazinyl, 1,2-diazinyl, 1,2-diazolyl, 1,4-diazanaphthalenyl, acridinyl, furo[3,2-b]pyridinyl, furo[3,2-c]pyridinyl, furo[2,3-c]pyridinyl, 6H-thieno[2,3-b]pyrrolyl, thieno[3,2-c]pyridinyl, thieno[2,3-c]pyrimidinyl, 1H-pyrrolo[2,3-b]pyridinyl, 1H-pyrrolo[2,3-c]pyridinyl, 1H-pyrrolo[3,2-b]pyridinyl, pyrrolo[1,2-a]pyrazinyl, pyrrolo[1,2-b]pyridazinyl, pyrazolo[1,5-a]pyridinyl, pyrazolo[1,5-a]pyrazinyl, imidazo[1,2-a]pyridinyl, 3H-imidazo[4,5-b]pyridinyl, imidazo[1,2-a]pyrimidinyl, imidazo[1,2-c]pyrimidinyl, imidazo[1,2-b]pyridazinyl, imidazo[1,2-a]pyrazinyl, imidazo[2,1-b][1,3]thiazolyl, imidazo[2,1-b][1,3,4]thiadiazolyl, [1,2,4]triazolo[1,5-a]pyridinyl, [1,2,4]triazolo[4,3-a]pyridinyl and the like. A heteroaryl radical is optionally substituted on a carbon or nitrogen atom ring member with substituent species as described herein where allowed by available valences.

As used herein, the term “heterocyclyl” generally refers to a saturated or partially unsaturated monocyclic, bicyclic or polycyclic carbon atom ring structure radical in which one or more carbon atom ring members have been replaced, where allowed by structural stability, with a heteroatom, such as an O, S or N atom, including, but not limited to, oxiranyl, oxetanyl, azetidyl, tetrahydrofuranlyl, pyrrolinyl, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, isoxazolinyl, isoxazolidinyl, isothiazolinyl, isothiazolidinyl, oxazolinyl, oxazolidinyl, thiazolinyl, thiazolidinyl, triazolinyl, triazolidinyl, oxadiazolinyl, oxadiazolidinyl, thiadiazolinyl, thiadiazolidinyl, tetrazolinyl, tetrazolidinyl, pyranlyl, dihydro-2H-pyranlyl, thiopyranlyl, 1,3-dioxanlyl, 1,2,5,6-tetrahydropyridinyl, 1,2,3,6-tetrahydropyridinyl, piperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, 1,4-diazepanyl, 1,3-benzodioxolyl (also referred to as benzo[d][1,3]diox-

olyl), 1,4-benzodioxanyl, 2,3-dihydro-1,4-benzodioxinyl (also referred to as 2,3-dihydrobenzo[b][1,4]dioxinyl), hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-b]pyrrol-(1H)-yl, hexahydropyrrolo[3,4-b]pyrrol-(2H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-b]pyrrol-(2H)-yl, hexahydropyrrolo[3,4-c]pyrrol-(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-c]pyrrol-(1H)-yl, (3aR,6aR)-hexahydropyrrolo[3,4-c]pyrrol-(1H)-yl, octahydro-5H-pyrrolo[3,2-c]pyridinyl, octahydro-6H-pyrrolo[3,4-b]pyridinyl, (4aR,7aR)-octahydro-6H-pyrrolo[3,4-b]pyridinyl, (4aR,7aR)-octahydro-6H-pyrrolo[3,4-b]pyridinyl, hexahydropyrrolo[1,2-c]pyrazin-(1H)-yl, (7R,8aR)-hexahydropyrrolo[1,2-c]pyrazin-(1H)-yl, (8aR)-hexahydropyrrolo[1,2-c]pyrazin-(1H)-yl, (8aR)-hexahydropyrrolo[1,2-c]pyrazin-(1H)-yl, (8aR)-octahydro-pyrrolo[1,2-c]pyrazin-(1H)-yl, (8aR)-octahydro-pyrrolo[1,2-c]pyrazin-(1H)-yl, hexahydropyrrolo[1,2-c]pyrazin-(2H)-one, octahydro-2H-pyrido[1,2-c]pyrazinyl, 3-azabicyclo[3.1.0]hexyl, (1R,5S)-3-azabicyclo[3.1.0]hexyl, 8-azabicyclo[3.2.1]octyl, (1R,5S)-8-azabicyclo[3.2.1]octyl, 8-azabicyclo[3.2.1]oct-2-enyl, (1R,5S)-8-azabicyclo[3.2.1]oct-2-enyl, 9-azabicyclo[3.3.1]nonyl, (1R,5S)-9-azabicyclo[3.3.1]nonyl, 2,5-diazabicyclo[2.2.1]heptyl, (1S,4S)-2,5-diazabicyclo[2.2.1]heptyl, 2,5-diazabicyclo[2.2.2]octyl, 3,8-diazabicyclo[3.2.1]octyl, (1R,5S)-3,8-diazabicyclo[3.2.1]octyl, 1,4-diazabicyclo[3.2.2]nonyl, azaspiro[3.3]heptyl, 2,6-diazaspiro[3.3]heptyl, 2,7-diazaspiro[3.5]nonyl, 5,8-diazaspiro[3.5]nonyl, 2,7-diazaspiro[4.4]nonyl, 6,9-diazaspiro[4.5]decyl and the like. A heterocyclyl radical is optionally substituted on a carbon or nitrogen atom ring member with substituent species as described herein where allowed by available valences.

As used herein, the term “C₁₋₈alkoxy-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-O—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkoxy-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-O—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-O—C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-NH—C₁₋₈alkyl-O—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-N(C₁₋₈alkyl-O—C₁₋₈alkyl)₂.

As used herein, the term “(C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-N(C₁₋₈alkyl)(C₁₋₈alkyl-O—C₁₋₈alkyl).

As used herein, the term “C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl-O—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl-O—C₁₋₈alkyl)₂.

As used herein, the term “(C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl)(C₁₋₈alkyl-O—C₁₋₈alkyl).

As used herein, the term “C₁₋₈alkoxy-carbonyl” refers to a radical of the formula: —C(O)—O—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkoxy-carbonyl-C₂₋₈alkenyl” refers to a radical of the formula: —C₂₋₈alkenyl-C(O)—O—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkoxy-carbonyl-amino” refers to a radical of the formula: —NH—C(O)—O—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkyl-amino-C₂₋₈alkenyl” refers to a radical of the formula: —C₂₋₈alkenyl-NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino-C₂₋₈alkenyl” refers to a radical of the formula: —C₂₋₈alkenyl-N(C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkyl-amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-N(C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkyl-amino-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-N(C₁₋₈alkyl)₂.

As used herein, the term “(C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-NH—C₁₋₈alkyl)₂.

As used herein, the term “[C₁₋₈alkyl)₂-amino-C₁₋₈alkyl]₂-amino” refers to a radical of the formula: —N[C₁₋₈alkyl-N(C₁₋₈alkyl)₂]₂.

As used herein, the term “(C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-NH—C₁₋₈alkyl).

As used herein, the term “[C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)[C₁₋₈alkyl-N(C₁₋₈alkyl)₂].

As used herein, the term “C₁₋₈alkyl-amino-C₂₋₈alkynyl” refers to a radical of the formula: —C₂₋₈alkynyl-NH—C₁₋₈alkyl.

As used herein, the term “(C₁₋₈alkyl)₂-amino-C₂₋₈alkynyl” refers to a radical of the formula: —C₂₋₈alkynyl-N(C₁₋₈alkyl)₂.

As used herein, the term “C₁₋₈alkyl-carbonyl” refers to a radical of the formula: —C(O)—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkyl-carbonyl-amino” refers to a radical of the formula: —NH—C(O)—C₁₋₈alkyl.

As used herein, the term “C₁₋₈alkyl-thio” refers to a radical of the formula: —S—C₁₋₈alkyl.

As used herein, the term “amino-C₂₋₈alkenyl” refers to a radical of the formula: —C₂₋₈alkenyl-NH₂.

As used herein, the term “amino-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-NH₂.

As used herein, the term “amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH₂.

As used herein, the term “amino-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-NH₂.

As used herein, the term “(amino-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-NH₂)₂.

As used herein, the term “(amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-NH₂).

As used herein, the term “amino-C₂₋₈alkynyl” refers to a radical of the formula: —C₂₋₈alkynyl-NH₂.

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As used herein, the term “aryl-C₁₋₈alkoxy-carbonyl” refers to a radical of the formula: —C(O)—O—C₁₋₈alkyl-aryl.

As used herein, the term “aryl-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-aryl.

As used herein, the term “aryl-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-aryl.

As used herein, the term “(aryl-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-aryl)₂.

As used herein, the term “(aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-aryl).

As used herein, the term “aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl-aryl.

As used herein, the term “(aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl-aryl)₂.

As used herein, the term “(aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl)(C₁₋₈alkyl-aryl).

As used herein, the term “aryl-amino” refers to a radical of the formula: —NH-aryl.

As used herein, the term “aryl-amino-carbonyl” refers to a radical of the formula: —C(O)—NH-aryl.

As used herein, the term “aryl-sulfonyloxy-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-O—SO₂-aryl.

As used herein, the term “benzoxo-carbonyl” refers to a radical of the formula: —C(O)O—CH₂-phenyl.

As used herein, the term “C₃₋₁₄cycloalkyl-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-C₃₋₁₄cycloalkyl.

As used herein, the term “C₃₋₁₄cycloalkyl-amino” refers to a radical of the formula: —NH—C₃₋₁₄cycloalkyl.

As used herein, the term “C₃₋₁₄cycloalkyl-oxy” refers to a radical of the formula: —O—C₃₋₁₄cycloalkyl.

As used herein, the term “halo” or “halogen” generally refers to a halogen atom radical, including fluoro, chloro, bromo and iodo.

As used herein, the term “halo-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-halo, wherein C₁₋₈alkyl is partially or completely substituted with one or more halogen atoms where allowed by available valences.

As used herein, the term “halo-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-halo, wherein C₁₋₈alkyl is partially or completely substituted with one or more halogen atoms where allowed by available valences.

As used herein, the term “halo-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-halo.

As used herein, the term “(halo-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-halo).

As used herein, the term “(halo-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-halo)₂.

As used herein, the term “heteroaryl-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-heteroaryl.

As used herein, the term “heteroaryl-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-heteroaryl.

As used herein, the term “heteroaryl-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-heteroaryl.

As used herein, the term “(heteroaryl-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-heteroaryl)₂.

As used herein, the term “(heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-heteroaryl).

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As used herein, the term “heteroaryl-C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl-heteroaryl.

As used herein, the term “(heteroaryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl-heteroaryl)₂.

As used herein, the term “(heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl)(C₁₋₈alkyl-heteroaryl).

As used herein, the term “heteroaryl-amino” refers to a radical of the formula: —NH-heteroaryl.

As used herein, the term “heterocyclyl-C₁₋₈alkoxy” refers to a radical of the formula: —O—C₁₋₈alkyl-heterocyclyl.

As used herein, the term “heterocyclyl-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-heterocyclyl.

As used herein, the term “heterocyclyl-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-heterocyclyl.

As used herein, the term “(heterocyclyl-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-heterocyclyl)₂.

As used herein, the term “(heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-heterocyclyl).

As used herein, the term “heterocyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl-heterocyclyl.

As used herein, the term “(heterocyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-MC₁₋₈alkyl-heterocyclyl).

As used herein, the term “(heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-N(C₁₋₈alkyl)(C₁₋₈alkyl-heterocyclyl).

As used herein, the term “heterocyclyl-amino” refers to a radical of the formula: —NH-heterocyclyl.

As used herein, the term “(heterocyclyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(heterocyclyl).

As used herein, the term “heterocyclyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH-heterocyclyl.

As used herein, the term “heterocyclyl-carbonyl” refers to a radical of the formula: —C(O)-heterocyclyl.

As used herein, the term “heterocyclyl-carbonyl-oxy” refers to a radical of the formula: —O—C(O)-heterocyclyl.

As used herein, the term “heterocyclyl-oxy” refers to a radical of the formula: —O-heterocyclyl.

As used herein, the term “hydroxy” refers to a radical of the formula: —OH.

As used herein, the term “hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-O—C₁₋₈alkyl-OH.

As used herein, the term “hydroxy-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-OH, wherein C₁₋₈alkyl is partially or completely substituted with one or more hydroxy radicals where allowed by available valences.

As used herein, the term “hydroxy-C₁₋₈alkyl-amino” refers to a radical of the formula: —NH—C₁₋₈alkyl-OH.

As used herein, the term “(hydroxy-C₁₋₈alkyl)₂-amino” refers to a radical of the formula: —N(C₁₋₈alkyl-OH)₂.

As used herein, the term “(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino” refers to a radical of the formula: —N(C₁₋₈alkyl)(C₁₋₈alkyl-OH).

As used herein, the term “hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl” refers to a radical of the formula: —C₁₋₈alkyl-NH—C₁₋₈alkyl-OH.

As used herein, the term “(hydroxy- C_{1-8} alkyl) $_2$ -amino- C_{1-8} alkyl” refers to a radical of the formula: $-C_{1-8}alkyl-N(C_{1-8} alkyl-OH)_2$.

As used herein, the term “(hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl) amino- C_{1-8} alkyl” refers to a radical of the formula: $-C_{1-8} alkyl-N(C_{1-8}alkyl)(C_{1-8}alkyl-OH)$.

As used herein, the term “hydroxy- C_{1-8} alkyl-amino- C_{1-8} alkoxy” refers to a radical of the formula: $-O-C_{1-8}alkyl-NH-C_{1-8}alkyl-OH$.

As used herein, the term “(hydroxy- C_{1-8} alkyl) $_2$ -amino- C_{1-8} alkoxy” refers to a radical of the formula: $-O-C_{1-8}alkyl-N(C_{1-8}alkyl-OH)_2$.

As used herein, the term “(hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl) amino- C_{1-8} alkoxy” refers to a radical of the formula: $-O-C_{1-8}alkyl-N(C_{1-8}alkyl)(C_{1-8}alkyl-OH)$.

As used herein, the term “hydroxy- C_{1-8} alkyl-amino- C_{1-8} alkyl-amino” refers to a radical of the formula: $-NH-C_{1-8}alkyl-NH-C_{1-8}alkyl-OH$.

As used herein, the term “(hydroxy- C_{1-8} alkyl-amino- C_{1-8} alkyl) $_2$ -amino” refers to a radical of the formula: $-N(C_{1-8}alkyl-NH-C_{1-8}alkyl-OH)_2$.

As used herein, the term “(hydroxy- C_{1-8} alkyl) $_2$ -amino- C_{1-8} alkyl-amino” refers to a radical of the formula: $-NH-C_{1-8}alkyl-N(C_{1-8}alkyl-OH)_2$.

As used herein, the term “(hydroxy- C_{1-8} alkyl-amino- C_{1-8} alkyl)(C_{1-8} alkyl)amino” refers to a radical of the formula: $-N(C_{1-8}alkyl)(C_{1-8}alkyl-NH-C_{1-8}alkyl-OH)$.

As used herein, the term “[hydroxy- C_{1-8} alkyl) $_2$ -amino- C_{1-8} alkyl](C_{1-8} alkyl)amino” refers to a radical of the formula: $-N(C_{1-8}alkyl)[C_{1-8}alkyl-N(C_{1-8}alkyl-OH)_2]$.

As used herein, the term “(hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl) amino- C_{1-8} alkyl-amino” refers to a radical of the formula: $-NH-C_{1-8}alkyl-N(C_{1-8}alkyl, C_{1-8}alkyl-OH)$.

As used herein, the term “(hydroxy- C_{1-8} alkyl)(C_{1-8} alkyl) amino- C_{1-8} alkyl](C_{1-8} alkyl)amino” refers to a radical of the formula: $-N(C_{1-8}alkyl)[C_{1-8}alkyl-N(C_{1-8}alkyl)(C_{1-8}alkyl-OH)]$.

As used herein, the term “substituent” means positional variables on the atoms of a core molecule that are attached at a designated atom position, replacing one or more hydrogen atoms on the designated atom, provided that the atom of attachment does not exceed the available valence or shared valences, such that the substitution results in a stable compound. Accordingly, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds. It should also be noted that any carbon as well as heteroatom with a valence level that appears to be unsatisfied as described or shown herein is assumed to have a sufficient number of hydrogen atom(s) to satisfy the valences described or shown.

For the purposes of this description, where one or more substituent variables for a compound of Formula (I) encompass functionalities incorporated into a compound of Formula (I), each functionality appearing at any location within the disclosed compound may be independently selected, and as appropriate, independently and/or optionally substituted.

As used herein, the terms “independently selected,” or “each selected” refer to functional variables in a substituent list that may be attached more than once on the structure of a core molecule, where the pattern of substitution at each occurrence is independent of the pattern at any other occurrence. Further, the use of a generic substituent on a core structure for a compound provided herein is understood to include the replacement of the generic substituent with specie substituents that are included within the particular genus, e.g., aryl may be independently replaced with phenyl or naphthalenyl (also referred to as naphthyl) and the like, such that the

resulting compound is intended to be included within the scope of the compounds described herein.

As used herein, the term “each instance of” when used in a phrase such as “. . . aryl, aryl- C_{1-8} alkyl, heterocyclyl and heterocyclyl- C_{1-8} alkyl, wherein each instance of aryl and heterocyclyl is optionally substituted with one or two substituents,” is intended to include optional, independent substitution on each of the aryl and heterocyclyl rings and on the aryl and heterocyclyl portions of aryl- C_{1-8} alkyl and heterocyclyl- C_{1-8} alkyl.

As used herein, the term “optionally substituted” means that the specified substituent variables, groups, radicals or moieties represent the scope of the genus and may be independently chosen as needed to replace one or more hydrogen atoms on the designated atom of attachment of a core molecule.

As used herein, the terms “stable compound” or “stable structure” mean a compound that is sufficiently robust to be isolated to a useful degree of purity from a reaction mixture and formulations thereof into an efficacious therapeutic agent.

Compound names provided herein were obtained using ACD Labs Index Name software provided by ACD Labs and/or ChemDraw Ultra software provided by Cambridge-Soft®. When the compound name disclosed herein conflicts with the structure depicted, the structure shown will supercede the use of the name to define the compound intended. Nomenclature for substituent radicals defined herein may differ slightly from the chemical name from which they are derived; one skilled in the art will recognize that the definition of the substituent radical is intended to include the radical as found in the chemical name.

The term “SMN,” unless otherwise specified herein, refers to the human SMN1 gene, DNA or RNA, and/or human SMN2 gene, DNA or RNA. In a specific embodiment, the term “SMN1” refers to the human SMN1 gene, DNA or RNA. In another specific embodiment, the term “SMN2” refers to the human SMN2 gene, DNA or RNA.

Nucleic acid sequences for the human SMN1 and SMN2 genes are known in the art. For nucleic acid sequences of human SMN1, see, e.g., GenBank Accession Nos. DQ894095, NM_000344, NM_022874, and BC062723. For nucleic acid sequences of human SMN2, see, e.g., NM_022875, NM_022876, NM_022877, NM_017411, DQ894734 (Life Technologies, Inc. (formerly Invitrogen), Carlsbad, Calif.), BC000908, BC070242, CR595484, CR598529, CR609539, U21914, and BC015308.

The SMN1 gene can be found on the forward strand of human chromosome 5 from approximately nucleotide 70,220,768 to approximately nucleotide 70,249,769. The approximate locations of exons 6, 7 and 8 and introns 6 and 7 of SMN1 on human chromosome 5 are as follows:

70,241,893 to 70,242,003 exon 6;
70,242,004 to 70,247,767 intron 6;
70,247,768 to 70,247,821 exon 7;
70,247,822 to 70,248,265 intron 7; and,
70,248,266 to 70,248,839 exon 8.

The SMN2 gene can be found on the forward strand of human chromosome 5 from approximately nucleotide 69,345,350 to approximately nucleotide 69,374,349.

The approximate locations of exons 6, 7 and 8 and introns 6 and 7 of SMN2 on human chromosome 5 are as follows:

69,366,468 to 69,366,578 exon 6;
69,366,579 to 69,372,347 intron 6;
69,372,348 to 69,372,401 exon 7;
69,372,402 to 69,372,845 intron 7; and,
69,372,846 to 69,373,419 exon 8.

In specific embodiments, the nucleotide sequences delineated above for exons 6, 7 and 8 and introns 6 and 7 of SMN1 are used in the SMN1 minigene nucleic acid constructs described herein. In other specific embodiments, the nucleotide sequences of exons 6, 7 and 8 and introns 6 and 7 of SMN2 in the examples provided herein are used in the SMN2 minigene nucleic acid constructs described herein.

The term "Smn" or "Smn protein," unless otherwise specified herein, refers to a human Smn protein that contains the amino acid residues encoded by exons 1 through 7 of the SMN1 gene and/or SMN2 gene. In a specific embodiment, the Smn protein is stable and functional in vitro and/or in vivo as assessed by methods known to one of skill in the art. In another specific embodiment, the Smn protein is the full-length protein encoded by the human SMN1 gene and/or SMN2 gene. In another specific embodiment, the Smn protein has the amino acid sequence found at GenBank Accession No. NP_000335, AAC50473.1, AAA66242.1, or NP_059107.

As used herein, the term "enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene," and analogous terms, unless otherwise specified herein, refers to the inclusion of the complete, intact, non-truncated sequence of exon 7 of SMN2 into the mature mRNA that is transcribed from the SMN2 gene (i.e., resulting in the production of full-length SMN2 mRNA) in vitro and/or in vivo, as assessed by methods known to one of skill in the art, such that increased levels of Smn protein are produced from the SMN2 gene in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that increased expression of stable and functional Smn protein is produced from the SMN2 gene in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that expression of the fusion protein encoded by the minigene is increased in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that expression of Smn protein produced from the SMN2 gene in a subject (e.g., an animal model for SMA or a human subject or an SMA patient) in need thereof is increased.

As used herein, the term "enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene," and analogous terms, unless otherwise specified herein, refers to the inclusion of the complete, intact, non-truncated sequence of exon 7 of SMN1 into the mature mRNA that is transcribed from the SMN1 gene (i.e., resulting in the production of full-length SMN1 mRNA) in vitro and/or in vivo, as assessed by methods known to one of skill in the art, such that increased levels of Smn protein are produced from the SMN1 gene in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that increased expression of stable and functional Smn protein is produced from the SMN1 gene in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that expression of the fusion protein encoded by the minigene is increased in vitro and/or in vivo, as assessed by methods known to one of skill in the art; or, that expression of Smn protein produced from the SMN1 gene in a subject (e.g., an animal model for SMA or a human subject) in need thereof is increased.

As used herein, the term "substantial change" in the context of the amount of mRNA means that the amount of mRNA does not change by a statistically significant amount, e.g., a p value less than a value selected from 0.1, 0.05, 0.01, 0.005, 0.001, 0.0005, 0.0001, 0.00005 or 0.00001.

As used herein, the terms "subject" and "patient" are used interchangeably to refer to an animal or any living organism having sensation and the power of voluntary movement, and which requires for its existence oxygen and organic food.

Nonlimiting examples include members of the human, equine, porcine, bovine, rattus, murine, canine and feline species. In some embodiments, the subject is a mammal or a warm-blooded vertebrate animal. In certain embodiments, the subject is a non-human animal. In specific embodiments, the subject is a human. In one specific embodiment, the subject is a human SMA patient.

As used herein, the term "elderly human" refers to a human 65 years old or older.

As used herein, the term "human adult" refers to a human that is 18 years or older.

As used herein, the term "human child" refers to a human that is 1 year to 18 years old.

As used herein, the term "human infant" refers to a newborn to 1 year old year human.

As used herein, the term "human toddler" refers to a human that is 1 year to 3 years old.

Compound Forms

As used herein, the terms "a compound of Formula (Ia)," "a compound of Formula (Ia1)," "a compound of Formula (Ia2)," "a compound of Formula (II)," "a compound of Formula (IIa)," "a compound of Formula (IIa1)," "a compound of Formula (IIa2)," "a compound of Formula (III)," "a compound of Formula (IIIa)," "a compound of Formula (IIIa1)," "a compound of Formula (IIIa2)," "a compound of Formula (IV)," "a compound of Formula (IVa)," "a compound of Formula (IVa1)," "a compound of Formula (IVa2)," "a compound of Formula (V)," "a compound of Formula (Va)," "a compound of Formula (Va1)" and "a compound of Formula (Va2)" each refer to subgenera of the compound of Formula (I) or a form thereof and are defined herein.

Rather than repeat embodiments for the various subgenera of the compound of Formula (I), in certain embodiments, the term "a compound of Formula (I) or a form thereof" is used to inclusively refer to a compound of Formula (Ia) or a form thereof, a compound of Formula (Ia1) or a form thereof, a compound of Formula (Ia2) or a form thereof, a compound of Formula (II) or a form thereof, a compound of Formula (IIa) or a form thereof, a compound of Formula (IIa1) or a form thereof, a compound of Formula (IIa2) or a form thereof, a compound of Formula (III) or a form thereof, a compound of Formula (IIIa) or a form thereof, a compound of Formula (IIIa1) or a form thereof, a compound of Formula (IIIa2) or a form thereof, a compound of Formula (IV) or a form thereof, a compound of Formula (IVa) or a form thereof, a compound of Formula (IVa1) or a form thereof, a compound of Formula (IVa2) or a form thereof, "a compound of Formula (V) or a form thereof," "a compound of Formula (Va) or a form thereof," "a compound of Formula (Va1) or a form thereof" and "a compound of Formula (Va2) or a form thereof" either separately or together.

Thus, embodiments and references to "a compound of Formula (I)" are intended to be inclusive of compounds of Formula (Ia), Formula (Ia1), Formula (Ia2), Formula (II), Formula (IIa), Formula (IIa1), Formula (IIa2), Formula (III), Formula (IIIa), Formula (IIIa1), Formula (IIIa2), Formula (IV), Formula (IVa), Formula (IVa1), Formula (IVa2), Formula (V), Formula (Va), Formula (Va1) and Formula (Va2).

As used herein, the term "form" means a compound of Formula (I) selected from a free acid, free base, salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer, or tautomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a selected from a salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a selected from a free acid, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a selected from a free base, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a free acid, free base or salt thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is an isotopologue thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a stereoisomer, racemate, enantiomer or diastereomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a tautomer thereof.

In certain embodiments described herein, the form of the compound of Formula (I) is a pharmaceutically acceptable form.

In certain embodiments described herein, the compound of Formula (I) or a form thereof is isolated for use.

As used herein, the term "isolated" means the physical state of a compound of Formula (I) or a form thereof after being isolated and/or purified from a synthetic process (e.g., from a reaction mixture) or natural source or combination thereof according to an isolation or purification process or processes described herein or which are well known to the skilled artisan (e.g., chromatography, recrystallization and the like) in sufficient purity to be characterizable by standard analytical techniques described herein or well known to the skilled artisan.

As used herein, the term "protected" means that a functional group on a compound of Formula (I) is in a form modified to preclude undesired side reactions at the protected site when the compound is subjected to a reaction. Suitable protecting groups will be recognized by those with ordinary skill in the art as well as by reference to standard textbooks such as, for example, T. W. Greene et al, *Protective Groups in Organic Synthesis* (1991), Wiley, New York.

Prodrugs of a compound of Formula (I) or a form thereof are also contemplated herein.

As used herein, the term "prodrug" means that a functional group on a compound of Formula (I) is in a form (e.g., acting as an active or inactive drug precursor) that is transformed in vivo to yield an active or more active compound of Formula (I) or a form thereof. The transformation may occur by various mechanisms (e.g., by metabolic and/or non-metabolic chemical processes), such as, for example, by hydrolysis and/or metabolism in blood, liver and/or other organs and tissues. A discussion of the use of prodrugs is provided by V. J. Stella, et. al., "Biotechnology: Pharmaceutical Aspects, Prodrugs: Challenges and Rewards," American Association of Pharmaceutical Scientists and Springer Press, 2007.

In one example, when a compound of Formula (I) or a form thereof contains a carboxylic acid functional group, a prodrug can comprise an ester formed by the replacement of the hydrogen atom of the acid group with a functional group such as alkyl and the like. In another example, when a compound of Formula (I) or a form thereof contains an alcohol functional group, a prodrug can be formed by the replacement of the hydrogen atom of the alcohol group with a functional group such as alkyl or substituted carbonyl and the like. In another example, when a compound of Formula (I) or a form thereof contains an amine functional group, a prodrug can be formed by the replacement of one or more amine hydrogen

atoms with a functional group such as alkyl or substituted carbonyl. In another example, when a compound of Formula (I) or a form thereof contains a hydrogen substituent, a prodrug can be formed by the replacement of one or more hydrogen atoms with an alkyl substituent.

Pharmaceutically acceptable prodrugs of compounds of Formula (I) or a form thereof include those compounds substituted with one or more of the following groups: carboxylic acid esters, sulfonate esters, amino acid esters phosphonate esters, mono-, di- or triphosphate esters or alkyl substituents where appropriate. As described herein, it is understood by a person of ordinary skill in the art that one or more of such substituents may be used to provide a compound of Formula (I) or a form thereof for use as a prodrug.

The compounds of Formula (I) can form salts which are intended to be included within the scope of this description. Reference to a compound of Formula (I) herein is understood to include reference to salts thereof, unless otherwise indicated. The term "salt(s)", as employed herein, denotes acidic salts formed with inorganic and/or organic acids, as well as basic salts formed with inorganic and/or organic bases. In addition, when a compound of Formula (I) contains both a basic moiety, such as, but not limited to a pyridine or imidazole, and an acidic moiety, such as, but not limited to a carboxylic acid, zwitterions ("inner salts") may be formed and are included within the term "salt(s)" as used herein.

The term "pharmaceutically acceptable salt(s)", as used herein, means those salts of compounds described herein that are safe and effective (i.e., non-toxic, physiologically acceptable) for use in mammals and that possess biological activity, although other salts are also useful. Salts of the compounds of Formula (I) may be formed, for example, by reacting a compound of Formula (I) with an amount of acid or base, such as an equivalent or stoichiometric amount, in a medium such as one in which the salt precipitates or in an aqueous medium followed by lyophilization.

Pharmaceutically acceptable salts include one or more salts of acidic or basic groups present in compounds described herein. Embodiments of acid addition salts include, and are not limited to, acetate, acid phosphate, ascorbate, benzoate, benzenesulfonate, bisulfate, bitartrate, borate, butyrate, chloride, citrate, camphorate, camphorsulfonate, ethanesulfonate, formate, fumarate, gentisinate, gluconate, glucuronate, glutamate, hydrobromide, hydrochloride, dihydrochloride, hydroiodide, isonicotinate, lactate, maleate, methanesulfonate, naphthalenesulfonate, nitrate, oxalate, pamoate, pantothenate, phosphate, propionate, saccharate, salicylate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate (also known as tosylate), trifluoroacetate salts and the like. One or more embodiments of acid addition salts include a chloride, hydrochloride, dihydrochloride, trihydrochloride, hydrobromide, acetate, diacetate or trifluoroacetate salt. More particular embodiments include a chloride, hydrochloride, dihydrochloride, hydrobromide or trifluoroacetate salt.

Additionally, acids which are generally considered suitable for the formation of pharmaceutically useful salts from basic pharmaceutical compounds are discussed, for example, by P. Stahl et al, Camille G. (eds.) *Handbook of Pharmaceutical Salts. Properties, Selection and Use.* (2002) Zurich: Wiley-VCH; S. Berge et al, *Journal of Pharmaceutical Sciences* (1977) 66(1) 1-19; P. Gould, *International J. of Pharmaceutics* (1986) 33, 201-217; Anderson et al, *The Practice of Medicinal Chemistry* (1996), Academic Press, New York; and in *The Orange Book* (see, website for Food & Drug Administration, Washington, D.C.). These disclosures are incorporated herein by reference thereto.

Suitable basic salts include, but are not limited to, aluminum, ammonium, calcium, lithium, magnesium, potassium, sodium, zinc, and diethanolamine salts. Certain compounds described herein can also form pharmaceutically acceptable salts with organic bases (for example, organic amines) such as, but not limited to, dicyclohexylamines, tert-butyl amines and the like, and with various amino acids such as, but not limited to, arginine, lysine and the like. Basic nitrogen-containing groups may be quarternized with agents such as lower alkyl halides (e.g., methyl, ethyl, and butyl chlorides, bromides and iodides), dialkyl sulfates (e.g., dimethyl, diethyl, and dibutyl sulfates), long chain halides (e.g., decyl, lauryl, and stearyl chlorides, bromides and iodides), aralkyl halides (e.g., benzyl and phenethyl bromides), and others.

All such acid salts and base salts are intended to be pharmaceutically acceptable salts within the scope of the description herein and all such acid and base salts are considered equivalent to the free forms of the corresponding compounds for the purposes described herein.

Compounds of Formula I and forms thereof may further exist in a tautomeric form. All such tautomeric forms are contemplated herein as part of the present description.

The compounds of Formula (I) may contain asymmetric or chiral centers, and, therefore, may exist in different stereoisomeric forms. The present description is intended to include all stereoisomeric forms of the compounds of Formula (I) as well as mixtures thereof, including racemic mixtures.

The compounds of Formula (I) described herein may include one or more chiral centers, and as such may exist as racemic mixtures (R/S) or as substantially pure enantiomers and diastereomers. The compounds may also exist as substantially pure (R) or (S) enantiomers (when one chiral center is present). In one embodiment, the compounds of Formula (I) described herein are (S) isomers and may exist as enantiomerically pure compositions substantially comprising only the (S) isomer. In another embodiment, the compounds of Formula (I) described herein are (R) isomers and may exist as enantiomerically pure compositions substantially comprising only the (R) isomer. As one of skill in the art will recognize, when more than one chiral center is present, the compounds of Formula (I) described herein may also include portions described as an (R,R), (R,S), (S,R) or (S,S) isomer, as defined by IUPAC Nomenclature Recommendations.

As used herein, the term "substantially pure" refers to compounds consisting substantially of a single isomer in an amount greater than or equal to 90%, in an amount greater than or equal to 92%, in an amount greater than or equal to 95%, in an amount greater than or equal to 98%, in an amount greater than or equal to 99%, or in an amount equal to 100% of the single isomer.

In one aspect, a compound of Formula (I) is a substantially pure (S) enantiomer present in an amount greater than or equal to 90%, in an amount greater than or equal to 92%, in an amount greater than or equal to 95%, in an amount greater than or equal to 98%, in an amount greater than or equal to 99%, or in an amount equal to 100%.

In one aspect, a compound of Formula (I) is a substantially pure (R) enantiomer present in an amount greater than or equal to 90%, in an amount greater than or equal to 92%, in an amount greater than or equal to 95%, in an amount greater than or equal to 98%, in an amount greater than or equal to 99%, or in an amount equal to 100%.

As used herein, a "racemate" is any mixture of isometric forms that are not "enantiomerically pure", including mixtures such as, without limitation, in a ratio of about 50/50, about 60/40, about 70/30, about 80/20, about 85/15 or about 90/10.

In addition, the present description embraces all geometric and positional isomers. For example, if a compound of Formula (I) incorporates a double bond or a fused ring, both the cis- and trans-forms, as well as mixtures, are embraced within the scope of the description herein.

Diastereomeric mixtures can be separated into their individual diastereomers on the basis of their physical chemical differences by methods well known to those skilled in the art, such as, for example, by chromatography and/or fractional crystallization. Enantiomers can be separated by use of chiral HPLC column or other chromatographic methods known to those skilled in the art.

Enantiomers can also be separated by converting the enantiomeric mixture into a diastereomeric mixture by reaction with an appropriate optically active compound (e.g., chiral auxiliary such as a chiral alcohol or Mosher's acid chloride), separating the diastereomers and converting (e.g., hydrolyzing) the individual diastereomers to the corresponding pure enantiomers. Also, some of the compounds of Formula (I) may be atropisomers (e.g., substituted biaryls) and are considered part of this description.

All stereoisomer forms (for example, geometric isomers, optical isomers, positional isomers and the like) of the present compounds (including salts, solvates, esters and prodrugs and transformed prodrugs thereof) which may exist due to asymmetric carbons on various substituents, including enantiomeric forms (which may exist even in the absence of asymmetric carbons), rotameric forms, atropisomers, diastereomeric forms and regioisomeric forms are contemplated within the scope of the description herein. For example, if a compound of Formula (I) incorporates a double bond or a fused ring, both the cis- and trans-forms, as well as mixtures thereof, are embraced within the scope of the description herein. Also, for example, all keto-enol and imine-enamine tautomeric forms of the compounds are included in the description herein. Individual stereoisomers of the compounds of Formula (I) described herein may, for example, be substantially free of other isomers, or may be present in a racemic mixture, as described supra.

The use of the terms "salt," "prodrug" and "transformed prodrug" are intended to equally apply to the salts, prodrugs and transformed prodrugs of all contemplated isotopologues, stereoisomers, racemates or tautomers of the instant compounds.

The term "isotopologue" refers to isotopically-enriched compounds which are identical to those recited herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds described herein include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, fluorine and chlorine, such as H^2 , H^3 , C^{13} , C^{14} , N^{15} , O^{18} , O^{17} , P^{31} , P^{32} , S^{35} , F^{18} , Cl^{35} and Cl^{36} , respectively, each of which is also within the scope of this description.

Certain isotopically-enriched compounds described herein (e.g., those labeled with H^3 and C^{14}) are useful in compound and/or substrate tissue distribution assays. Tritiated (i.e., H^3) and carbon-14 (i.e., C^{14}) isotopes are particularly preferred for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., "deuterium enriched") may afford certain therapeutic advantages resulting from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements) and hence may be preferred in some circumstances. Isotopically-enriched compounds of Formula (I) can generally be prepared using procedures known to persons of ordinary skill in the art by

substituting an appropriate isotopically-enriched reagent for a non-isotopically-enriched reagent.

When the compounds are enriched with deuterium, the deuterium-to-hydrogen ratio on the deuterated atoms of the molecule substantially exceeds the naturally occurring deuterium-to-hydrogen ratio.

An embodiment described herein may include an isotopologue form of the compound of Formula (I), wherein the isotopologue is substituted on one or more atom members of the compound of Formula (I) with one or more deuterium atoms in place of one or more hydrogen atoms.

An embodiment described herein may include a compound of Formula (I) and forms thereof, wherein a carbon atom may have from 1 to 3 hydrogen atoms optionally replaced with deuterium.

One or more compounds described herein may exist in unsolvated as well as solvated forms with pharmaceutically acceptable solvents such as water, ethanol, and the like, and the description herein is intended to embrace both solvated and unsolvated forms.

As used herein, the term "solvate" means a physical association of a compound described herein with one or more solvent molecules. This physical association involves varying degrees of ionic and covalent bonding, including hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. As used herein, "solvate" encompasses both solution-phase and isolatable solvates. Non-limiting examples of suitable solvates include ethanolates, methanolates, and the like.

One or more compounds described herein may optionally be converted to a solvate. Preparation of solvates is generally known. A typical, non-limiting process involves dissolving a compound in a desired amount of the desired solvent (organic or water or mixtures thereof) at a higher than ambient temperature, and cooling the solution at a rate sufficient to form crystals which are then isolated by standard methods. Analytical techniques such as, for example infrared spectroscopy, show the presence of the solvent (or water) in the crystals as a solvate (or hydrate).

As used herein, the term "hydrate" means a solvate wherein the solvent molecule is water.

Polymorphic crystalline and amorphous forms of the compounds of Formula (I), and of the salts, solvates, esters and prodrugs of the compounds of Formula (I), are further intended to be included in the scope of the compounds described herein.

Compound Uses

Compounds of Formula (I) or a form thereof that enhance inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene are described herein. Such compounds of Formula (I) or a form thereof have been shown to enhance the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene using the assays described herein (see Biological example section, *infra*). Accordingly, compounds of Formula (I) or a form thereof have utility as enhancers for the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene.

Compounds of Formula (I) or a form thereof for enhancing inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene are described herein. Such compounds of Formula (I) or a form thereof may enhance inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene using, e.g., an SMN1 minigene assay. Accordingly, compounds of Formula (I) or a form thereof may have utility as enhancers for the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene.

In one aspect, provided herein are methods for modulating the inclusion of exon 7 of SMN2 into RNA transcribed from the SMN2 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein are methods for modulating the inclusion of exon 7 of SMN2 into RNA transcribed from the SMN2 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof that modulates the expression of an SMN2 minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In another embodiment, the minigene is the minigene described in Biological Example 1, *infra*. The human cell can be contacted with a compound of Formula (I) or a form thereof in vitro and/or in vivo, e.g., in a non-human animal or in a human. In a specific embodiment, the human cell is from or in a human. In another specific embodiment, the human cell is from or in a human SMA patient. In another specific embodiment, the human cell is from or in a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In another embodiment, the human cell is a human cell from a human SMA patient. In certain embodiments, the human cell is from a cell line, such as GM03813, GM00232, GM09677, and/or GM23240 (available from Coriell Institute). In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In a specific embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof. In another embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof that enhances the expression of an SMN2 minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In another embodiment, the minigene is the minigene described in Biological Example 1, *infra*. The human cell can be contacted with a compound of Formula (I) or a form thereof in vitro and/or in vivo, e.g., in a non-human animal or in a human. In a specific embodiment, the human cell is from or in a human. In another specific embodiment, the human cell is from or in a human SMA patient. In another specific embodiment, the human cell is from or in a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In another embodiment, the human cell is a human cell from a human SMA patient. In certain embodiments, the human cell is from a cell line, such as GM03813, GM00232, GM09677, and/or GM23240 (available from Coriell Institute). In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein are methods for enhancing the inclusion of exon 7 of SMN1 into RNA transcribed from the SMN1 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein are methods for

enhancing the inclusion of exon 7 of SMN1 into RNA transcribed from the SMN1 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof. In another specific embodiment, provided herein are methods for enhancing the inclusion of exon 7 of SMN1 into RNA transcribed from the SMN1 gene, comprising contacting a human cell with a compound of Formula (I) or a form thereof that modulates the expression of an SMN1 minigene described in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. The human cell can be contacted with a compound of Formula (I) or a form thereof in vitro and/or in vivo, e.g., in a non-human animal or in a human. In a specific embodiment, the human cell is from or in a human. In another specific embodiment, the human cell is from or in a human SMA patient. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In specific embodiments, provided herein are methods for enhancing the inclusion of exon 7 of SMN1 and SMN2 into RNA transcribed from the SMN1 and SMN2 genes, comprising contacting a human cell with a compound of Formula (I) or a form thereof. The human cell can be contacted with a compound of Formula (I) or a form thereof in vitro and/or in vivo, e.g., in a non-human animal or in a human. In a specific embodiment, the human cell is from or in a human. In another specific embodiment, the human cell is from or in a human SMA patient. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein is a method for modulating the inclusion of exon 7 of SMN2 into RNA transcribed from the SMN2 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for modulating the inclusion of exon 7 of SMN2 into RNA transcribed from the SMN2 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof that modulates the expression of an SMN2 minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In another embodiment, the minigene is the minigene described in Biological Example 1, *infra*. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In a specific embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof. In another specific embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof that enhances the expression of an SMN2 minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Pub-

lication No. 20110086833. In another embodiment, the minigene is the minigene described in Biological Example 1, *infra*. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein is a method for enhancing the inclusion of exon 7 of SMN1 into RNA transcribed from the SMN1 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN1 into RNA transcribed from the SMN1 gene, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof that modulates the expression of an SMN1 minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In one embodiment, the minigene is a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In specific embodiments, provided herein is a method for enhancing the inclusion of exon 7 of SMN1 and SMN2 into RNA transcribed from the SMN1 and SMN2 genes, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein is a method for increasing the amount of Smn protein, comprising contacting a human cell with a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for increasing the amount of Smn protein, comprising contacting a human cell with a compound of Formula (I) that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. In another specific embodiment, provided herein is a method for increasing the amount of Smn protein, comprising contacting a human cell with a compound of Formula (I) that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene. The human cell can be contacted with a compound of Formula (I) or a form thereof in vitro and/or in vivo, e.g., in a non-human animal or in a human. In a specific embodiment, the human cell is from or in a human. In another specific embodiment, the human cell is from or in a human SMA patient. In another specific embodiment, the human cell is from or in a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In another embodiment, the human cell is a human cell from a human SMA patient. In certain embodiments, the human cell is from a cell line, such as GM03813, GM00232, GM09677, and/or GM23240 (available from Coriell Institute). In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein is a method for increasing the amount of Smn protein, comprising administering to a non-human animal model for SMA a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for increasing the amount of Smn protein, comprising administering to a non-human animal model for SMA a compound of Formula (I) that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in, e.g., a cell-based or cell-free assay, such as described in the Biological Examples, *infra*. In another specific embodiment, provided herein is a method for increasing

the amount of Smn protein, comprising administering to a non-human animal model for SMA a compound of Formula (I) that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene in, e.g., a cell-based or cell-free assay.

In one embodiment, the compound of Formula (I) enhances the expression of a minigene described herein or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In a specific embodiment, the compound of Formula (I) enhances the expression of a minigene described in the Examples of International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In another specific embodiment, the compound of Formula (I) enhances the expression of a minigene described in Biological Example 1, *infra*. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In one embodiment, provided herein is the use of a compound of Formula (I) or a form thereof for the preparation of a medicament that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. In another embodiment, provided herein is the use of a compound of Formula (I) or a form thereof for the preparation of a medicament that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, thereby increasing expression of Smn protein in a human subject in need thereof. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in an assay described herein (see, e.g., the Biological Examples, *infra*). In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In one embodiment, provided herein is the use of a compound of Formula (I) or a form thereof for the preparation of a medicament that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene. In another embodiment, provided herein is the use of a compound of Formula (I) or a form thereof for the preparation of a medicament that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene, thereby increasing expression of Smn protein in a human subject in need thereof. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein are methods for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in a human subject in need thereof, comprising administering to the human subject an effective amount of a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in a human subject in need thereof, comprising administering to the human subject an effective amount a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*). In specific embodiments, the effective amount of the compound of Formula (I) or a form thereof is administered to the human subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier, excipient or diluent. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in an assay described herein (see, e.g., the Biological Examples, *infra*). In a specific embodiment,

the human subject is a human SMA patient. In another specific embodiment, the human subject is a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein are methods for enhancing the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene in a human subject in need thereof, comprising administering to the human subject an effective amount of a compound of Formula (I) or a form thereof. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene in an assay described in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833. In specific embodiments, the effective amount of the compound of Formula (I) or a form thereof is administered to the human subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier, excipient or diluent. In a specific embodiment, the human subject is a human SMA patient. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein is a method for enhancing the inclusion of exon 7 of SMN1 and SMN2 into mRNA that is transcribed from the SMN1 and SMN2 genes in a human subject in need thereof, comprising administering to the human subject an effective amount a compound of Formula (I) or a form thereof. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 into mRNA that is transcribed from the SMN1 gene in an assay(s) described in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833 (see, e.g., the Examples in those publications), each of which is incorporated herein by reference in its entirety. In specific embodiments, the effective amount of the compound of Formula (I) or a form thereof is administered to the human subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier, excipient or diluent. In a specific embodiment, the human subject is a human SMA patient. In another specific embodiment, the human subject is a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein are methods for enhancing the expression of Smn protein in a human subject in need thereof, comprising administering to the human subject an effective amount of a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for enhancing the expression of Smn protein in a human subject in need thereof, comprising administering to the human subject an effective amount a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. In another specific embodiment, provided herein is a method for enhancing the expression of Smn protein in a human subject in need thereof, comprising administering to the human subject an effective amount a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene. In specific embodiments, the effective amount of the compound of Formula (I) or a form thereof is administered to the human subject in a pharmaceutical composition comprising a pharmaceutically acceptable carrier, excipient or diluent. In a particular embodiment, the

compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene in an assay described herein (see, e.g., the Biological Examples, *infra*) or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833 (see, e.g., the Examples in those publications), each of which is incorporated herein by reference in its entirety.

In a specific embodiment, the human subject is a human SMA patient. In another specific embodiment, the human subject is a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the teleomeric copy of the SMN1 gene in both chromosomes, resulting in a loss of SMN1 gene function. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another embodiment, provided herein is the use of a compound of Formula (I) or a form thereof for the preparation of a medicament that enhances expression of Smn protein in a human subject in need thereof. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*). In another embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*) or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833 (see, e.g., the Examples in those publications), each of which is incorporated herein by reference in its entirety. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In another aspect, provided herein are methods for treating spinal muscular atrophy (SMA), comprising administering to a subject an effective amount of a compound of Formula (I) or a form thereof. In a specific embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject an effective amount of a compound of Formula (I) or a form thereof. In another specific embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising an effective amount of a compound of Formula (I) or a form thereof, and a pharmaceutically acceptable carrier, excipient or diluent. In one embodiment, the compound is a compound of Formula (I) or a form thereof.

In another embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject an effective amount of a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene. In a specific embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising an effective amount of a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, and a pharmaceutically acceptable carrier, excipient or diluent.

In another specific embodiment, provided herein is a method for treating SMA in a human subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising an effective amount of a compound of Formula (I) or a form thereof that enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene, and a pharmaceutically

acceptable carrier, excipient or diluent. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in an assay described herein (see, e.g., the Biological Examples, *infra*). In another embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*) or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833 (see, e.g., the Examples in those publications), each of which is incorporated herein by reference in its entirety. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In another embodiment, provided herein is the use of a compound of Formula (I) or a form thereof in the manufacture of a medicament for treating SMA in a human subject in need thereof. In a particular embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*). In another embodiment, the compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene as determined in an assay described herein (see, e.g., the Biological Examples, *infra*) or in International Publication No. WO2009151546 or U.S. Patent Application Publication No. 20110086833 (see, e.g., the Examples in those publications), each of which is incorporated herein by reference in its entirety. In a specific embodiment, the compound is a compound of Formula (I) or a form thereof.

In an embodiment of a use or method provided herein, compounds of Formula (I) or a form thereof are used in combination with one or more additional agents. A compound(s) of Formula (I) or a form thereof can be administered to a subject or contacted with a cell prior to, concurrently with, or subsequent to administering to the subject or contacting the cell with an additional agent(s). A compound(s) of Formula (I) or a form thereof and an additional agent(s) can be administered to a subject or contacted with a cell in single composition or different compositions. In a specific embodiment, a compound(s) of Formula (I) or a form thereof is used in combination with gene replacement of SMN1 (using, e.g., viral delivery vectors). In another specific embodiment, a compound(s) of Formula (I) or a form thereof are used in combination with cell replacement using differentiated SMN1^{+/+} and/or SMN2^{+/+} stem cells. In another specific embodiment, a compound(s) of Formula (I) or a form thereof are used in combination with cell replacement using differentiated SMN1^{+/+} stem cells. In another specific embodiment, a compound(s) of Formula (I) or a form thereof are used in combination with cell replacement using differentiated SMN2^{+/+} stem cells. In another specific embodiment, a compound(s) of Formula (I) or a form thereof are used in combination with acylarubicin. In another specific embodiment, a compound(s) of Formula (I) or a form thereof are used in combination with a transcription activator such as a histone deacetylase ("HDAC") inhibitor (e.g., butyrate, valproic acid, and hydroxyurea), and mRNA stabilizers (e.g., mRNA decapping inhibitor RG3039 from Repligen).

In one embodiment, provided herein is the use of compounds of Formula (I) or a form thereof in combination with supportive therapy, including respiratory, nutritional or rehabilitation care.

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In certain embodiments, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) has a therapeutic effect and/or beneficial effect. In a specific embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in one, two or more of the following effects: (i) reduces or ameliorates the severity of SMA; (ii) delays onset of SMA; (iii) inhibits the progression of SMA; (iv) reduces hospitalization of a subject; (v) reduces hospitalization length for a subject; (vi) increases the survival of a subject; (vii) improves the quality of life of a subject; (viii) reduces the number of symptoms associated with SMA; (ix) reduces or ameliorates the severity of a symptom(s) associated with SMA; (x) reduces the duration of a symptom associated with SMA; (xi) prevents the recurrence of a symptom associated with SMA; (xii) inhibits the development or onset of a symptom of SMA; and/or (xiii) inhibits the progression of a symptom associated with SMA.

Symptoms of SMA include muscle weakness, poor muscle tone, weak cry, weak cough, limpness or a tendency to flop, difficulty sucking or swallowing, difficulty breathing, accumulation of secretions in the lungs or throat, clenched fists with sweaty hand, flickering/vibrating of the tongue, head often tilted to one side, even when lying down, legs that tend to be weaker than the arms, legs frequently assuming a "frog legs" position, feeding difficulties, increased susceptibility to respiratory tract infections, bowel/bladder weakness, lower-than-normal weight, inability to sit without support, failure to walk, failure to crawl, and hypotonia, areflexia, and multiple congenital contractures (arthrogryposis) associated with loss of anterior horn cells.

In a specific embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in one, two or more of the following effects: (i) a reduction in the loss of muscle strength; (ii) an increase in muscle strength; (iii) a reduction in muscle atrophy; (iv) a reduction in the loss of motor function; (v) an increase in motor neurons; (vi) a reduction in the loss of motor neurons; (vii) protection of SMN deficient motor neurons from degeneration; (ix) an increase in motor function; (x) an increase in pulmonary function; and/or (xi) a reduction in the loss of pulmonary function.

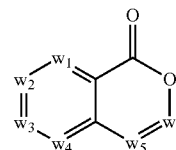
In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant or a human toddler to sit up. In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to stand up unaided. In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to walk unaided. In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to run unaided. In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to breathe unaided. In another embodiment, treating SMA with a com-

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pound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to turn during sleep unaided. In another embodiment, treating SMA with a compound of Formula (I) or a form thereof (alone or in combination with an additional agent) results in the functional ability or helps retain the functional ability for a human infant, a human toddler, a human child or a human adult to swallow unaided.

In certain embodiments, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot, to determine whether a compound of Formula (I) or a form thereof enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from an SMN1 and/or SMN2 gene. In some embodiments, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 8, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification, Northern blot or Southern blot, or a pharmaceutical or assay kit as described infra, to monitor patient responses to a compound of Formula (I) or a form thereof.

In a specific embodiment, a compound of Formula (I):



(I)

or a form thereof is used in accordance with a method described herein, wherein:

w_1 is C— R_b or N;

w_2 and w_6 are C— R_1 or C— R_2 ;

w_3 , w_4 and w_5 are C— R_a or N;

wherein one of w_2 and w_6 is C— R_1 and the other is C— R_2 , provided that, when w_2 is C— R_1 , then w_6 is C— R_2 ; or, when w_2 is C— R_2 , then w_6 is C— R_1 ; and,

wherein any one, two or three of the remaining w_1 , w_3 , w_4 and w_5 may simultaneously be N;

R_1 is C_{1-8} alkyl, amino, C_{1-8} alkyl-amino, $(C_{1-8}$ alkyl) $_2$ -amino, C_{1-8} alkoxy- C_{1-8} alkyl-amino, $(C_{1-8}$ alkoxy- C_{1-8} alkyl) $_2$ -amino, $(C_{1-8}$ alkoxy- C_{1-8} alkyl)(C_{1-8} alkyl)amino, amino- C_{1-8} alkyl, C_{1-8} alkyl-amino- C_{1-8} alkyl, $(C_{1-8}$ alkyl) $_2$ -amino- C_{1-8} alkyl, C_{1-8} alkoxy- C_{1-8} alkyl-amino- C_{1-8} alkyl, $(C_{1-8}$ alkoxy- C_{1-8} alkyl) $_2$ -amino- C_{1-8} alkyl, $(C_{1-8}$ alkoxy- C_{1-8} alkyl)(C_{1-8} alkyl)amino- C_{1-8} alkyl, amino- C_{1-8} alkyl-amino, (amino- C_{1-8} alkyl) $_2$ -amino, (amino- C_{1-8} alkyl)(C_{1-8} alkyl)amino, C_{1-8} alkyl-amino- C_{1-8} alkyl-amino, $(C_{1-8}$ alkyl-amino- C_{1-8} alkyl) $_2$ -amino, $(C_{1-8}$ alkyl-amino- C_{1-8} alkyl)(C_{1-8} alkyl)amino, $(C_{1-8}$ alkyl) $_2$ -amino- C_{1-8} alkyl-amino, $[(C_{1-8}$ alkyl) $_2$ -amino- C_{1-8} alkyl](C_{1-8} alkyl)amino, amino- C_{1-8} alkoxy, C_{1-8} alkyl-amino- C_{1-8} alkoxy, $(C_{1-8}$ alkyl) $_2$ -amino- C_{1-8} alkoxy, C_{1-8} alkoxy- C_{1-8} alkyl-amino- C_{1-8} alkoxy, C_{1-8} alkoxy- C_{1-8} alkyl-amino- C_{1-8} alkoxy, $(C_{1-8}$ alkoxy- C_{1-8} alkyl)(C_{1-8} alkyl)amino- C_{1-8} alkoxy, amino- C_{2-8} alkenyl, C_{1-8} alkyl-amino- C_{2-8} alkenyl, $(C_{1-8}$ alkyl) $_2$ -

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amino-C₂₋₈alkenyl, amino-C₂₋₈alkynyl, C₁₋₈alkyl-amino-C₂₋₈alkynyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkynyl, halo-C₁₋₈alkyl-amino, (halo-C₁₋₈alkyl)₂-amino, (halo-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl-amino, [(hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, [(hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, heterocyclyl, heterocyclyl-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkoxy, heterocyclyl-amino, (heterocyclyl)(C₁₋₈alkyl)amino, heterocyclyl-amino-C₁₋₈alkyl, heterocyclyl-C₁₋₈alkyl-amino, (heterocyclyl-C₁₋₈alkyl)₂-amino, (heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, heterocyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (heterocyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heterocyclyl-oxy, heterocyclyl-carbonyl, heterocyclyl-carbonyl-oxy, aryl-C₁₋₈alkyl-amino, (aryl-C₁₋₈alkyl)₂-amino, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, hetero aryl, hetero aryl-C₁₋₈alkyl, hetero aryl-C₁₋₈alkoxy, hetero aryl-amino, hetero aryl-C₁₋₈alkyl-amino, (heteroaryl-C₁₋₈alkyl)₂-amino, (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino, hetero aryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hetero aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl or (hetero aryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl;

wherein, each instance of heterocyclyl and heteroaryl is optionally substituted with one, two or three R₃ substituents and one additional, optional R₄ substituent; and, wherein, alternatively, each instance of heterocyclyl and heteroaryl is optionally substituted with one, two, three or four R₃ substituents;

R₂ is aryl, aryl-amino, aryl-amino-carbonyl, heterocyclyl, heteroaryl or heteroaryl-amino;

wherein, each instance of aryl, heterocyclyl and heteroaryl is optionally substituted with one, two or three R₆ substituents and one additional, optional R₇ substituent;

R_a is, in each instance, independently selected from hydrogen, halogen or C₁₋₈alkyl;

R_b is hydrogen, halogen, C₁₋₈alkyl or C₁₋₈alkoxy;

R₃ is, in each instance, independently selected from cyano, halogen, hydroxy, oxo, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkyl-carbonyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, C₁₋₈alkoxy-carbonyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, amino-C₁₋₈alkyl-amino, C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl]₂-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-carbonyl-amino, C₁₋₈alkoxy-carbonyl-amino, hydroxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkoxy-

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xy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino or (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino;

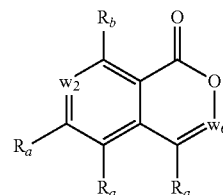
R₄ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-C₁₋₈alkyl, C₃₋₁₄cycloalkyl-amino, aryl-C₁₋₈alkyl, aryl-C₁₋₈alkoxy-carbonyl, aryl-sulfonyloxy-C₁₋₈alkyl, heterocyclyl or heterocyclyl-C₁₋₈alkyl; wherein, each instance of C₃₋₁₄cycloalkyl, aryl and heterocyclyl is optionally substituted with one, two or three R₅ substituents;

R₅ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio;

R₆ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, C₂₋₈alkenyl, halo-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio; and,

R₇ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-oxy, aryl, heterocyclyl or heteroaryl.

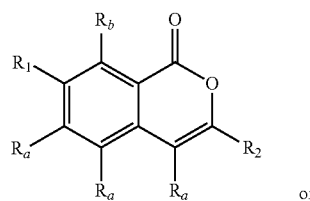
In one embodiment of the use described herein, the compound of Formula (I) is a compound selected from Formula (Ia):



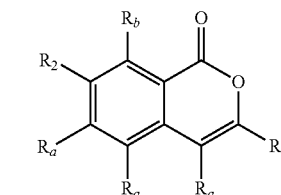
(Ia)

or a form thereof.

In another embodiment of the use described herein, the compound of Formula (Ia) is a compound selected from Formula (Ia1) or Formula (Ia2):



(Ia1)

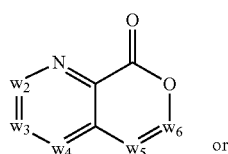
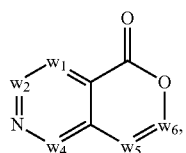
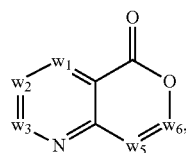


(Ia2)

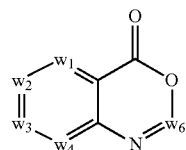
or a form thereof.

In one embodiment of the use described herein, the compound of Formula (I) is a compound selected from Formula (II), Formula (III), Formula (IV) or Formula (V):

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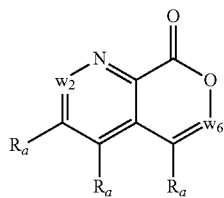
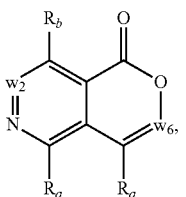
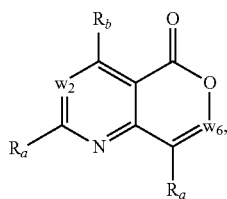


or



or a form thereof.

In another embodiment of the use described herein, the compound of Formula (II), Formula (III), Formula (IV) and Formula (V) is a compound selected from Formula (IIa), Formula (IIIa), Formula (IVa) and Formula (Va), respectively:



and

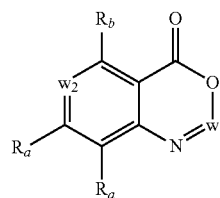
90

-continued

(II)

(Va)

5



10

(III)

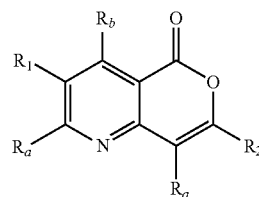
or a form thereof.

In another embodiment of the use described herein, the compound of Formula (IIa) is a compound selected from Formula (IIa1) or Formula (IIa2):

(IV)

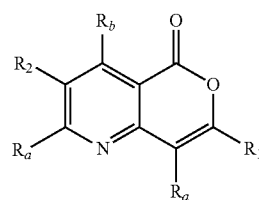
(IIa1)

20



(V)

25



(IIa2)

30

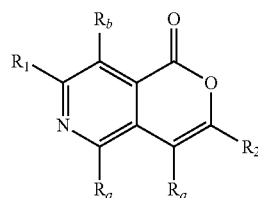
or a form thereof.

In another embodiment of the use described herein, the compound of Formula (IIIa) is a compound selected from Formula (IIIa1) or Formula (IIIa2):

(IIa)

(IIIa1)

45



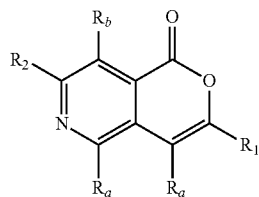
(IIIa)

50

or

(IIIa2)

55



(IVa)

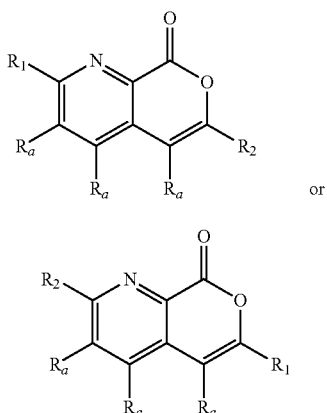
60

or a form thereof.

In another embodiment of the use described herein, the compound of Formula (IVa) is a compound selected from Formula (IVa1) or Formula (IVa2):

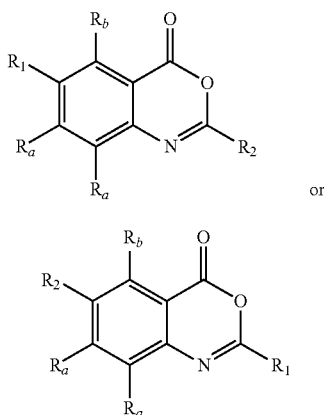
65

91



or a form thereof.

In another embodiment of the use described herein, the compound of Formula (Va) is a compound selected from Formula (Va1) or Formula (Va2):



or a form thereof.

Patient Population

In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject suffering from SMA. In other embodiments, a compound of Formula (I) or a form thereof, is administered to a subject predisposed or susceptible to SMA. In a specific embodiment, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human subject, wherein the human subject is a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In certain embodiments, the human subject is genotyped prior to administration of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof to determine whether the subject has an inactivating mutation or deletion in the teleomeric copy of the SMN1 gene in both chromosomes, which results in a loss of SMN1 gene function. In some embodiments, a compound of Formula (I) or a form thereof, or pharmaceutical composition thereof is administered to a subject with Type 0 SMA. In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject with

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(IVa1)

Type 1 SMA. In other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject with Type 2 SMA. In other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject with Type 3 SMA. In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject with Type 4 SMA.

(IVa2)

In certain embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject that will or might benefit from enhanced inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene. In specific embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a subject that will or may benefit from enhanced Smn protein expression.

In certain embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human that has an age in a range of from about 0 months to about 6 months old, from about 6 to about 12 months old, from about 6 to about 18 months old, from about 18 to about 36 months old, from about 1 to about 5 years old, from about 5 to about 10 years old, from about 10 to about 15 years old, from about 15 to about 20 years old, from about 20 to about 25 years old, from about 25 to about 30 years old, from about 30 to about 35 years old, from about 35 to about 40 years old, from about 40 to about 45 years old, from about 45 to about 50 years old, from about 50 to about 55 years old, from about 55 to about 60 years old, from about 60 to about 65 years old, from about 65 to about 70 years old, from about 70 to about 75 years old, from about 75 to about 80 years old, from about 80 to about 85 years old, from about 85 to about 90 years old, from about 90 to about 95 years old or from about 95 to about 100 years old.

(Va2)

In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human infant. In other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human toddler. In other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human child. In other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to a human adult. In yet other embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof is administered to an elderly human.

In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, an effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, a prophylactically effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, a therapeutically effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA.

In some embodiments, a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to an SMA patient to treat or ameliorate SMA.

In other embodiments, an effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to an SMA patient to treat or ameliorate SMA. In other embodiments, a prophylactically effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to an SMA patient to prevent advancement of SMA. In other embodiments, a therapeutically effective amount of a compound of Formula (I) or a form thereof, or a pharmaceutical composition thereof, is administered to an SMA patient to treat or ameliorate SMA.

In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject suffering from SMA. In other embodiments, a compound of Formula (I) or a form thereof, is administered to a subject predisposed or susceptible to SMA. In a specific embodiment, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human subject, wherein the human subject is a human SMA patient, wherein SMA is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function. In certain embodiments, the human subject is genotyped prior to administration of a compound of Formula (I) or a form thereof, or a medicament thereof to determine whether the subject has an inactivating mutation or deletion in the teleomeric copy of the SMN1 gene in both chromosomes, which results in a loss of SMN1 gene function. In some embodiments, a compound of Formula (I) or a form thereof, or medicament thereof is administered to a subject with Type 0 SMA. In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject with Type 1 SMA. In other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject with Type 2 SMA. In other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject with Type 3 SMA. In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject with Type 4 SMA.

In certain embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject that will or might benefit from enhanced inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene. In specific embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a subject that will or may benefit from enhanced Smn protein expression.

In certain embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human that has an age in a range of from about 0 months to about 6 months old, from about 6 to about 12 months old, from about 6 to about 18 months old, from about 18 to about 36 months old, from about 1 to about 5 years old, from about 5 to about 10 years old, from about 10 to about 15 years old, from about 15 to about 20 years old, from about 20 to about 25 years old, from about 25 to about 30 years old, from about 30 to about 35 years old, from about 35 to about 40 years old, from about 40 to about 45 years old, from about 45 to about 50 years old, from about 50 to about 55 years old, from about 55 to about 60 years old, from about 60 to about 65 years old, from about 65 to about 70 years old, from about 70 to about 75 years old, from about 75 to about 80 years old, from about 80 to about 85 years old, from about 85 to about 90 years old, from about 90 to about 95 years old or from about 95 to about 100 years old.

In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human infant. In other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human toddler. In other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human child. In other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a human adult. In yet other embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to an elderly human.

In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, an effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, a prophylactically effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA. In other embodiments, a therapeutically effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to a patient to prevent the onset of SMA in a patient at risk of developing SMA.

In some embodiments, a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to an SMA patient to treat or ameliorate SMA. In other embodiments, an effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to an SMA patient to treat or ameliorate SMA. In other embodiments, a prophylactically effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to an SMA patient to prevent advancement of SMA. In other embodiments, a therapeutically effective amount of a compound of Formula (I) or a form thereof, or a medicament thereof, is administered to an SMA patient to treat or ameliorate SMA.

Mode of Administration

When administered to a patient, a compound of Formula (I) or a form thereof is preferably administered as a component of a composition that optionally comprises a pharmaceutically acceptable carrier, excipient or diluent. The composition can be administered orally, or by any other convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal, and intestinal mucosa) and may be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, and can be used to administer the compound.

Methods of administration include but are not limited to parenteral, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, oral, sublingual, intranasal, intracerebral, intravaginal, transdermal, rectally, by inhalation, or topically, particularly to the ears, nose, eyes, or skin. The mode of administration is left to the discretion of the practitioner. In most instances, administration will result in the release of a compound into the bloodstream. In a specific embodiment, a compound is administered orally.

Dosage and Dosage Forms

The amount of a compound of Formula (I) or a form thereof that will be effective in the treatment of SMA depend, e.g., on the route of administration, the type of SMA, the general health of the subject, ethnicity, age, weight, and gender of the

subject, diet, time, and the severity of SMA, and should be decided according to the judgment of the practitioner and each patient's or subject's circumstances.

In specific embodiments, an "effective amount," "prophylactically effective amount" or "therapeutically effective amount" in the context of the administration of a compound of Formula (I) or a form thereof, or composition or medicament thereof refers to an amount of a compound of Formula (I) which has a therapeutic effect and/or beneficial effect. In certain specific embodiments, an "effective amount," "prophylactically effective amount" or "therapeutically effective amount" in the context of the administration of a compound of Formula (I) or a form thereof, or composition or medicament thereof results in one, two or more of the following effects: (i) reduces or ameliorates the severity of SMA; (ii) delays onset of SMA; (iii) inhibits the progression of SMA; (iv) reduces hospitalization of a subject; (v) reduces hospitalization length for a subject; (vi) increases the survival of a subject; (vii) improves the quality of life of a subject; (viii) reduces the number of symptoms associated with SMA; (ix) reduces or ameliorates the severity of a symptom(s) associated with SMA; (x) reduces the duration of a symptom associated with SMA; (xi) prevents the recurrence of a symptom associated with SMA; (xii) inhibits the development or onset of a symptom of SMA; and/or (xiii) inhibits the progression of a symptom associated with SMA. In certain embodiments, an effective amount of a compound of Formula (I) or a form thereof is an amount effective to enhance inclusion of exon 7 of SMN2 into SMN2 mRNA that is transcribed from the SMN2 gene and increases the levels of Snn protein produced from the SMN2 gene and thus producing a desired beneficial effect in a subject in need thereof. In some instances, the desired effect can be determined by analyzing or quantifying: (1) the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene; or (2) the levels of Snn protein produced from the SMN2 gene. Non-limiting examples of effective amounts of a compound of Formula (I) or a form thereof are described herein.

For example, the effective amount may be the amount required to treat SMA in a human subject in need thereof, or the amount required to enhance inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene in a human subject in need thereof, or the amount required to increase levels of Snn protein produced from the SMN2 gene in a human subject in need thereof. In a specific embodiment, the human subject is an SMA patient.

In general, the effective amount will be in a range of from about 0.001 mg/kg/day to about 500 mg/kg/day for a patient or subject having a weight in a range of between about 1 kg to about 200 kg. The typical adult subject is expected to have a median weight in a range of between about 70 and about 100 kg.

Within the scope of the present description, the "effective amount" of a compound of Formula (I) or a form thereof for use in the manufacture of a medicament, the preparation of a pharmaceutical kit or in a method for treating SMA in a human subject in need thereof, is intended to include an amount in a range of from about 0.001 mg to about 35,000 mg. In a specific embodiment, the human subject is an SMA patient.

The compositions described herein are formulated for administration to the subject via any drug delivery route known in the art. Nonlimiting examples include oral, ocular, rectal, buccal, topical, nasal, ophthalmic, subcutaneous, intramuscular, intravenous (bolus and infusion), intracerebral, transdermal, and pulmonary routes of administration.

Pharmaceutical Compositions

Embodiments described herein include the use of a compound of Formula (I) or a form thereof in a pharmaceutical composition. In a specific embodiment, described herein is the use of a compound of Formula (I) or a form thereof in a pharmaceutical composition for treating SMA in a human subject in need thereof comprising administering an effective amount of a compound of Formula (I) or a form thereof in admixture with a pharmaceutically acceptable excipient. In a specific embodiment, the human subject is an SMA patient.

A compound of Formula (I) or a form thereof may optionally be in the form of a composition comprising the compound or a form thereof and an optional carrier, excipient or diluent. Other embodiments provided herein include pharmaceutical compositions comprising an effective amount of a compound of Formula (I) or a form thereof and a pharmaceutically acceptable carrier, excipient, or diluent. In a specific embodiment, the pharmaceutical compositions are suitable for veterinary and/or human administration. The pharmaceutical compositions provided herein can be in any form that allows for the composition to be administered to a subject.

In a specific embodiment and in this context, the term "pharmaceutically acceptable carrier, excipient or diluent" means a carrier, excipient or diluent approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (e.g., Freund's adjuvant (complete and incomplete)), excipient, or vehicle with which a therapeutic agent is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a specific carrier for intravenously administered pharmaceutical compositions. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions.

Typical compositions and dosage forms comprise one or more excipients. Suitable excipients are well-known to those skilled in the art of pharmacy, and non limiting examples of suitable excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art including, but not limited to, the way in which the dosage form will be administered to a patient and the specific active ingredients in the dosage form. Further provided herein are anhydrous pharmaceutical compositions and dosage forms comprising one or more compounds of Formula (I) or a form thereof as described herein. The compositions and single unit dosage forms can take the form of solutions or syrups (optionally with a flavoring agent), suspensions (optionally with a flavoring agent), emulsions, tablets (e.g., chewable tablets), pills, capsules, granules, powder (optionally for reconstitution), taste-masked or sustained-release formulations and the like.

Pharmaceutical compositions provided herein that are suitable for oral administration can be presented as discrete dosage forms, such as, but are not limited to, tablets, caplets, capsules, granules, powder, and liquids. Such dosage forms contain predetermined amounts of active ingredients, and may be prepared by methods of pharmacy well known to those skilled in the art.

Examples of excipients that can be used in oral dosage forms provided herein include, but are not limited to, binders, fillers, disintegrants, and lubricants.

Biomarkers

In certain embodiments, the amount of mRNA that is transcribed from the SMN1 gene and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 is used as a biomarker for SMA. In certain embodiments, the amount of mRNA that is transcribed from the SMN1 gene and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 is used as a biomarker for SMA. In other embodiments, the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 is used as a biomarker for an SMA patient being treated with a compound, such as disclosed herein. In other embodiments, the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 is used as a biomarker for an SMA patient being treated with a compound, such as disclosed herein. In some embodiments, a change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and a corresponding change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 is a biomarker for a patient being treated with a compound, such as disclosed herein. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and a corresponding decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 after the administration of a compound (e.g., a compound of Formula (I) disclosed herein) indicates that the compound may be effective to treat SMA. In another specific embodiment, a decrease in the amount of mRNA that is transcribed from the SMN2 gene and includes exon 7 of SMN2 and a corresponding increase in the amount of mRNA that is transcribed from the SMN2 gene and does not include exon 7 of SMN2 after the administration of a compound (e.g., a compound of Formula (I) disclosed herein) indicates that the compound will not be effective to treat SMA. In accordance with these embodiments, an SMN primer(s) and/or an SMN probe described below can be used in assays, such as PCR (e.g., qPCR) and RT-PCR (e.g., RT-qPCR or endpoint RT-PCR) to assess and/or quantify the amount of mRNA that is transcribed from the SMN1 gene and/or SMN2 gene and does or does not include exon 7 of SMN1 and/or SMN2.

In one embodiment, provided herein are SMN primers and/or SMN probes (e.g., a forward primer having the nucleotide sequence of SEQ ID NO. 1, 7, 8, 11 or 13; and/or a reverse primer having the nucleotide sequence of SEQ ID NO. 9 or 12; and/or an SMN probe such as a SEQ ID NO. 3 or 10) for amplifying nucleic acids encoding or encoded by human SMN1 and/or SMN2. These primers can be used as primers in, e.g., RT-PCR (such as RT-PCR, endpoint RT-PCR and/or RT-qPCR as described herein or as known to one skilled in the art), PCR (such as qPCR) or rolling circle amplification, and as probes in hybridization assays, such as a Northern blot and/or a Southern blot assay. As utilized in the Biological Examples herein, endpoint RT-PCR is a reverse transcription-polymerase chain reaction that is carried out for a certain number of amplification cycles (or until starting materials are exhausted) following by a quantification of each

of the DNA products using, e.g., gel electrophoretic separation, staining with a fluorescent dye, quantification of fluorescence and the like.

SEQ ID NO. 1 hybridizes to DNA or RNA comprising nucleotides corresponding to nucleotides 22 to 40 of exon 7 of SMN1 and/or SMN2, SEQ ID NO. 2 hybridizes to DNA or RNA comprising nucleotides corresponding to nucleotides 4 to 26 of the firefly luciferase coding sequence; SEQ ID NO. 7 hybridizes to nucleic acid sequences (e.g., the sense strand of DNA) comprising nucleotides corresponding to nucleotides 32 to 54 of exon 7 of SMN1 and/or SMN2 and nucleotides 1 to 4 of exon 8 of SMN1 and/or SMN2, SEQ ID NO. 8 hybridizes to nucleic acid sequences (e.g., the sense strand of DNA) comprising nucleotides corresponding, in order, to nucleotides 87 to 111 of exon 7 of SMN1 and/or SMN2 and nucleotides 1 to 3 of exon 8 of SMN1 and/or SMN2, SEQ ID NO. 9 hybridizes to nucleic acid sequences (e.g., the antisense strand of DNA or RNA) comprising nucleotides corresponding to nucleotides 39 to 62 of exon 8 of SMN1 and/or SMN2, SEQ ID NO. 11 hybridizes to nucleic acid sequences (e.g., the sense strand of DNA) comprising nucleotides corresponding to nucleotides 43 to 63 of exon 6 of SMN1 and/or SMN2, SEQ ID NO. 12 hybridizes to nucleic acid sequences (e.g., the antisense strand of DNA or RNA) comprising nucleotides corresponding to nucleotides 51 to 73 of exon 8 of SMN1 and/or SMN2, and SEQ ID NO. 13 hybridizes to nucleic acid sequence (e.g., the sense strand of DNA) comprising nucleotides corresponding to nucleotides 22 to 46 of exon 6 of SMN1 and/or SMN2.

Accordingly, an oligonucleotide corresponding to SEQ ID NO. 9, 11, 12 and/or 13 can be used in an amplification reaction to amplify nucleic acids encoding or encoded by human SMN1 and/or SMN2 lacking exon 7 of human SMN1 and/or SMN2 and nucleic acid encoding or encoded by human SMN1 and/or SMN2 and includes exon 7 of human SMN1 and/or SMN2. In contrast, an oligonucleotide corresponding to SEQ ID NO. 8 in conjunction with a downstream reverse primer (e.g., SEQ ID NO. 9 or 12) can be used to amplify nucleic acids encoding or encoded by human SMN1 and/or SMN2 lacking exon 7 of human SMN1 and/or SMN2 and an oligonucleotide corresponding to SEQ ID NO. 1 and 7 in conjunction with a downstream reverse primer (e.g., SEQ ID NO. 9 or 12) can be used to amplify nucleic acids encoding or encoded by human SMN1 and/or human SMN2 and includes exon 7 of SMN1 and/or SMN2.

SEQ ID NO. 3 hybridizes to nucleic acid sequences (e.g., the sense strand of DNA) comprising nucleotides corresponding, in order, to nucleotides 50 to 54 of exon 7 of human SMN1 and/or SMN2 and nucleotides 1 to 21 of exon 8 of human SMN1 and/or SMN2, and SEQ ID NO. 10 hybridizes to nucleic acid sequences (e.g., the sense strand of DNA) comprising nucleotides corresponding to nucleotides 7 to 36 of exon 8 of human SMN1 and/or SMN2. SEQ ID NO. 3 is useful as a probe to detect mRNA that is transcribed from the minigene and includes exon 7 of SMN1 and/or SMN2, described herein or described in International Publication No. WO 2009151546 or U.S. Patent Application Publication No. 20110086833 (each of which is incorporated herein by reference in its entirety) and to detect mRNA that is transcribed from human SMN1 and/or SMN2 and includes exon 7 of SMN1 and/or SMN2. In addition, SEQ ID NO. 10 is useful as a probe to detect mRNA that is transcribed from the minigene and does or does not include exon 7 of SMN1 and/or SMN2 and to detect mRNA that is transcribed from human SMN1 and/or SMN2, described herein or as described in International Publication No. WO 2009151546 or U.S. Patent Appli-

cation Publication No. 20110086833, each of which is incorporated herein by reference in its entirety.

In a specific embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 11 or 13 and/or SEQ ID NO. 2, 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to determine whether a compound (e.g., a compound of Formula (I) or a form thereof) enhances the inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from an SMN1 and/or SMN2 gene.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in a patient sample. In a specific embodiment, the patient is an SMA patient.

In another embodiment, a primer and/or probe described below in the Biological Examples (e.g., SMN primers such as SEQ ID NO. 1, 7, 11 or 13 and/or SEQ ID NO. 9 or 12, and/or SMN probes such as a SEQ ID NO. 3 or 10) is used in an assay, such as RT-PCR, RT-qPCR, endpoint RT-PCR, PCR, qPCR, rolling circle amplification and, as applicable, Northern blot or Southern blot (e.g., an assay such as described below in the Biological Examples), to monitor a patient's response to a compound (e.g., a compound of Formula (I) or a form thereof). In a specific embodiment, the patient is an SMA patient.

A sample (e.g., a blood sample, PBMC sample, or tissue sample, such as a skin or muscle tissue sample) from a patient can be obtained using techniques known to one skilled in the art and the primers and/or probes described in the Biological Examples below can be used in assays (e.g., PCR, RT-PCR, RT-qPCR, qPCR, endpoint RT-PCR, rolling circle amplification, Northern blot and Southern blot) to determine the amount of mRNA that is transcribed from the SMN1 and/or SMN2 genes (e.g., the amount of mRNA that includes exon 7 of SMN2 transcribed from the SMN2 gene). A sample derived from a patient refers to a sample that is processed and/or manipulated after being obtained from the patient using techniques known to one skilled in the art. For example, a sample from a patient can be processed to, e.g., extract RNA, using techniques known to one of skill in the art. A sample from a patient can be processed to, e.g., extract RNA and the RNA is reversed transcribed to produce cDNA. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (b) detecting the amount of

mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In another specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and SMN2 genes, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and SMN2 genes. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

The amount of mRNA that is transcribed from the human SMN1 and SMN2 genes that includes exon 7 of SMN1 and SMN2 and the amount of mRNA that is transcribed from the human SMN1 and SMN2 genes and does not include exon 7 of SMN1 and SMN2 can be differentiated from each other by, e.g., size of the RNA or DNA fragment generated from SMN1 and SMN2 mRNA that includes exon 7 of SMN1 and SMN2 and from SMN1 and SMN2 mRNA that do not include exon 7 of SMN1 and SMN2.

In another specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In another specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with an SMN probe described below (e.g., SEQ ID NO. 3 or 10) along with applicable components, e.g., of an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR), rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a com-

pound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In another specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and SMN2 genes, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with an SMN probe described below (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR), rolling circle amplification and, as applicable, Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and SMN2 genes.

The amount of mRNA that is transcribed from the human SMN1 and SMN2 genes that includes exon 7 of SMN1 and SMN2 and the amount of mRNA that is transcribed from the human SMN1 and SMN2 genes and does not include exon 7 of SMN1 and SMN2 can be differentiated from each other by, e.g., size of the RNA or DNA fragment generated from SMN1 and SMN2 mRNA that includes exon 7 of SMN1 and SMN2 and from SMN1 and SMN2 mRNA that do not include exon 7 of SMN1 and SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In another specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with an SMN probe described below (e.g., SEQ ID NO. 10) along with applicable components for, e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR), rolling circle amplification, or Northern blot or Southern blot; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the

SMN1 and SMN2 genes, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe described herein (e.g., SEQ ID NO. 3 or 10) along with applicable components for e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, as applicable; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and SMN2 genes. In a specific embodiment, the patient is an SMA patient.

The amount of mRNA that is transcribed from the human SMN1 and SMN2 genes that includes exon 7 of SMN1 and SMN2 and the amount of mRNA that is transcribed from the human SMN1 and SMN2 genes that do not include exon 7 of SMN1 and SMN2 can be differentiated from each other by, e.g., size of the RNA or DNA fragment generated from SMN1 and SMN2 mRNA that includes exon 7 of SMN1 and SMN2 and from SMN1 and SMN2 mRNA that does not include exon 7 of SMN1 and SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, provided herein is a method for detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, comprising: (a) contacting a patient sample (e.g., blood sample or tissue sample) or a sample derived from a patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe described herein (e.g., SEQ ID NO. 10) along with applicable components for e.g., an RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2. In certain embodiments, the sample is from or derived from a patient administered a compound, such as a compound of Formula (I) or a form thereof as described herein. In a specific embodiment, the patient is an SMA patient.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that

the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 3 or 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an

SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 3 or 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more

after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA

that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for e.g., RT-PCR (e.g.,

endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of

SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration

of the compound, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a com-

pound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR) or PCR (e.g., qPCR), wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount

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of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for assessing an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not

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include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound, indicate that the SMN1 and/or patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is assessed 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that

the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of

1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 3 or 10) along with applicable components for e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) administering a compound to an

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SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 1, 7, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 3 or 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2, wherein (1) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a

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decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or

SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from a patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the

patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's responsiveness to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 8, 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to the administration of the compound or a certain number of doses of the compound, or a certain earlier date indicates that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40,

40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more

after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR), or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and

that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of

SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with an SMN probe (e.g., SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a

certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In a specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) contacting an SMA patient sample (e.g., blood sample or tissue sample) or a sample derived from an SMA patient (e.g., a blood sample or tissue sample that has been processed to extract RNA) with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification, wherein the sample is from or derived from an SMA patient administered a compound (e.g., a compound of Formula (I) or a form thereof as described herein); and (b) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date,

indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In another specific embodiment, provided herein is a method for monitoring an SMA patient's response to a compound, comprising: (a) administering a compound to an SMA patient; (b) contacting a sample (e.g., blood sample or tissue sample) obtained or derived from the patient with a forward SMN primer described below (e.g., SEQ ID NO. 11 or 13) and/or a reverse SMN primer described herein (e.g., SEQ ID NO. 9 or 12) and/or an SMN probe (SEQ ID NO. 10) along with applicable components for, e.g., RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification; and (c) detecting the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, wherein (1)(i) an increase in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) a decrease in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the

amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., from the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is responsive to the compound and that the compound may be or is beneficial and/or of therapeutic value to the patient; and (2)(i) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, and (ii) no change or no substantial change in the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in the patient sample relative to the amount of mRNA that is transcribed from the SMN2 gene and does not include exon 7 of SMN1 and/or SMN2 in an analogous sample (e.g., the same type of tissue sample) from the patient prior to administration of the compound or a certain number of doses of the compound, or a certain earlier date, indicate that the patient is not responsive to the compound and that the compound is not beneficial and/or of therapeutic value to the patient. In certain embodiments, the patient's response is monitored 1 hour, 2 hours, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 28 days, 1 month, 2 months, 3 months, 6 months, 9 months, 12 months or more after administration of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the patient has received 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored after the administration of 1-5, 5-10, 10-15, 15-20, 20-30, 30-40, 40-50, or 50-100 doses of a compound, such as a compound of Formula (I) or a form thereof as described herein. In some embodiments, the patient's response is monitored over a period of days, weeks, months or years during or after the continuous administration of a compound, such as a compound of Formula (I) or a form thereof as described herein.

In specific embodiments, SMA in a patient is caused by an inactivating mutation or deletion in the SMN1 gene on both chromosomes, resulting in a loss of SMN1 gene function.

Kits

In one aspect, provided herein are pharmaceutical or assay kits comprising an SMN primer or probe described herein, in one or more containers, and instructions for use. In one embodiment, a pharmaceutical or assay kit comprises, in a container, one or more SMN reverse primers (e.g., SEQ ID NO. 2, 9 and/or 12) and/or one or more SMN forward primers (SEQ ID NO. 1, 7, 8, 11 and/or 13)) and instructions for use. In another embodiment, a pharmaceutical or assay kit comprises, in one container, an SMN reverse primer (e.g., SEQ ID NO. 2, 9 or 12), an SMN forward primer (SEQ ID NO. 1, 7, 8, 11 or 13)) and instructions for use.

In one embodiment, a pharmaceutical or assay kit comprises, in separate containers, one SMN reverse primer (e.g., SEQ ID NO. 2, 9 or 12) in one container, another SMN forward primer (e.g., SEQ ID NO. 1, 7, 8, 11 or 13)) in another container, and instructions for use.

In certain embodiments, applicable components needed for a PCR (e.g., qPCR), RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR) or rolling circle amplification, such as polymerase, deoxynucleoside triphosphates, etc., are included in such kits. In some embodiments, components needed for hybridization are included in such kits. A pharmaceutical or assay kit containing such primers can be used in PCR and RT-PCR to, e.g., (i) assess whether a therapeutic agent (e.g., a compound of Formula (I) or a form thereof) enhances inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene, (ii) monitor the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 of SMN1 and/or SMN2 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, and/or (iii) monitor a subject's response to a therapeutic agent (e.g., a compound of Formula (I) or a form thereof). In other embodiments, the subject is a human subject. In other embodiments, the human subject is a human patient. In certain other embodiments, the human patient is a human SMA patient.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the sequence found in SEQ ID NO. 1, in a container, and the reverse primer with the sequence found in SEQ ID NO. 2, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by a human SMN1 minigene or human SMN2 minigene, such as described those described herein or in International Publication No. WO 2009151546 or U.S. Patent Application Publication No. 20110086833, each of which is incorporated herein by reference in its entirety. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 7, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In another specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 8, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by the endogenous human SMN2 gene. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 7, in a container, the forward primer with the nucleotide sequence found in SEQ ID NO. 8, in another container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2

genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 11, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 12, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 11, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 13, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 12, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 13, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 1, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 9, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these primers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In a specific embodiment, a pharmaceutical or assay kit comprises the forward primer with the nucleotide sequence found in SEQ ID NO. 1, in a container, and the reverse primer with the nucleotide sequence found in SEQ ID NO. 12, in another container. In certain embodiments, these primers are used in RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR), PCR (e.g., qPCR) or rolling circle amplification for amplifying nucleotide sequences encoded by endogenous human SMN1 and SMN2 genes. In other embodiments, these prim-

ers are used as probes in, e.g., hybridization assays, such as Southern blot or Northern blot.

In another embodiment, a pharmaceutical or assay kit comprises an SMN probe described herein (e.g., SEQ ID NO. 3 or 10), in one container. In other embodiments, the probe is used in, e.g., a hybridization assay, such as a Southern blot or Northern blot. In a specific embodiment, the probe is used in RT-qPCR or qPCR. In certain embodiments, components needed for a PCR (e.g., qPCR), RT-PCR (e.g., endpoint RT-PCR and/or RT-qPCR) or rolling circle amplification, such as polymerase, deoxynucleoside triphosphates, primers, etc., are included in such kits. In some embodiments, components needed for hybridization are included in such kits.

In one embodiment, a pharmaceutical or assay kit comprises an SMN reverse primer (e.g., SEQ ID NO. 2, 9 or 12) in one container, an SMN forward primer (e.g., SEQ ID NO. 1, 7, 8, 11 or 13) in another container, and an SMN probe (e.g., SEQ ID NO. 3 or 10) in another container, and instructions for use. In another embodiment, a pharmaceutical or assay kit comprises one or more SMN reverse primers (e.g., SEQ ID NO. 2, 9 and/or 12) in one container, one or more SMN forward primers (e.g., SEQ ID NO. 1, 7, 8, 11 and/or 13) in another container, and one or more SMN probe (e.g., SEQ ID NO. 3 and/or 10) in another container, and instructions for use.

In certain embodiments, components needed to run a PCR, RT-PCR or rolling circle amplification, such as polymerase, deoxynucleoside triphosphates, etc., are included in such kits. A pharmaceutical or assay kit containing such probes and/or primers can be used in PCR and RT-PCR to, e.g.,: (i) assess whether a therapeutic agent (e.g., a compound of Formula (I) or a form thereof) enhances inclusion of exon 7 of SMN1 and/or SMN2 into mRNA that is transcribed from the SMN1 and/or SMN2 gene, (ii) monitor the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and includes exon 7 and the amount of mRNA that is transcribed from the SMN1 and/or SMN2 gene and does not include exon 7 of SMN1 and/or SMN2, and/or (iii) monitor a subject's response to a therapeutic agent (e.g., a compound of Formula (I) or a form thereof). In other embodiments, the subject is a human subject. In other embodiments, the human subject is a human patient. In certain other embodiments, the human patient is a human SMA patient.

In another aspect, provided herein is a pharmaceutical kit comprising a compound of Formula (I) or a form thereof, in a container, and instructions for use of the compound or form thereof. In a specific embodiment, provided herein is a pharmaceutical kit comprising a pharmaceutical composition comprising a compound of Formula (I) or a form thereof and a pharmaceutically acceptable carrier, excipient or diluent, and instructions for use. In another specific embodiment, provided herein is a pharmaceutical kit comprising a pharmaceutical composition comprising an effective amount of a compound of Formula (I) or a form thereof and a pharmaceutically acceptable carrier, excipient or diluent, and instructions for use. In one embodiment, the instructions for use explain one, two or more of the following: the dose, route of administration, frequency of administration and side effects of administration of a compound of Formula (I) or a form thereof to a subject. In other embodiments, the subject is a human subject. In other embodiments, the human subject is a human patient. In certain other embodiments, the human patient is a human SMA patient.

General Synthetic Methods

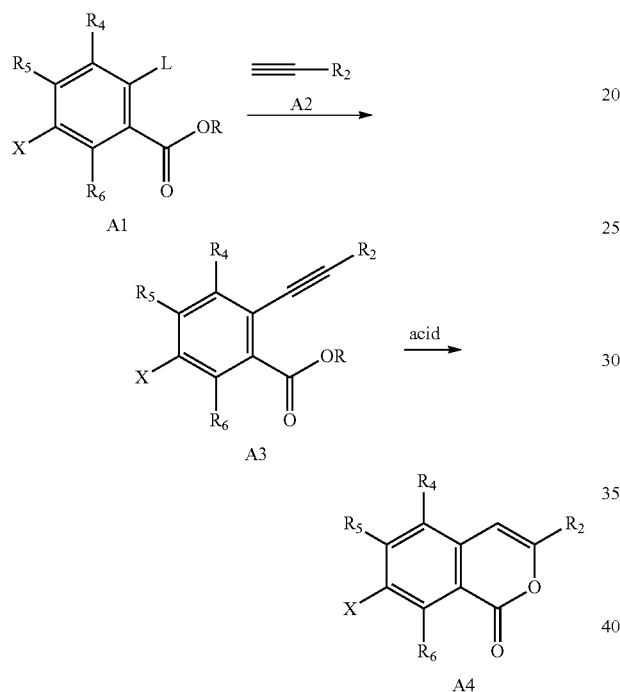
As disclosed herein, general methods for preparing the compounds of Formula (I) or a form thereof as described herein are available via standard, well-known synthetic meth-

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odology. Many of the starting materials are commercially available or, when not available, can be prepared using the routes described below using techniques known to those skilled in the art. The synthetic schemes provided herein comprise multiple reaction steps, each of which is intended to stand on its own and can be carried out with or without any preceding or succeeding step(s). In other words, each of the individual reactions steps of the synthetic schemes provided herein in isolation is contemplated.

Scheme A

Compounds of Formula (I), wherein R_2 is a monocyclic or bicyclic aryl, heterocyclyl or heteroaryl ring system, may be prepared as described in Scheme A below.

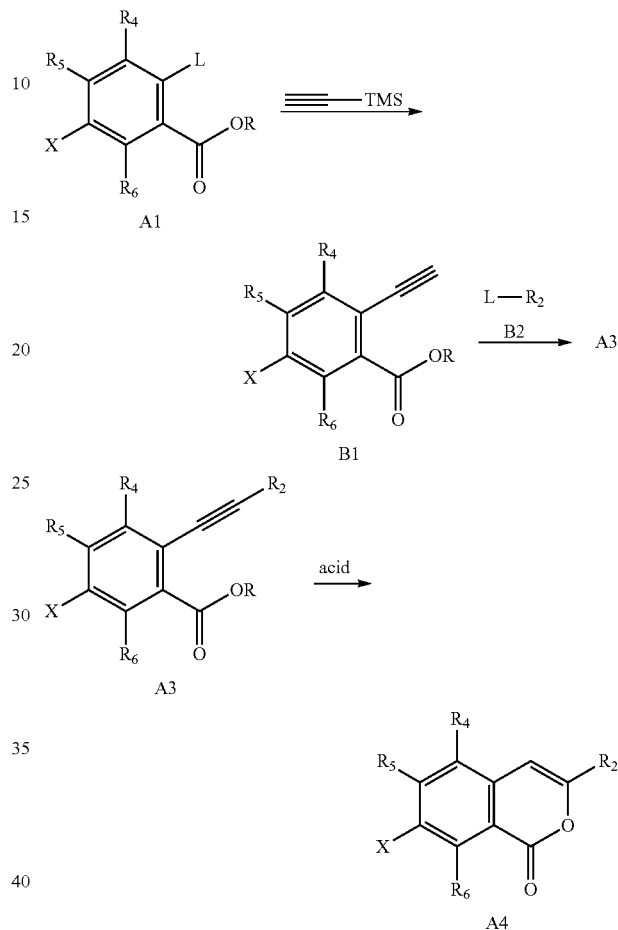


Compound A1 (where X represents various reactive groups, which may be used to provide a plurality of R_1 functional group substituents by reacting suitable starting materials with Compound A1, Compound A3 or Compound A4 using techniques known to a person of ordinary skill in the art and where L is a leaving group, such as halogen or trifluoromethylsulfonyloxy and the like) is reacted with Compound A2 in the presence of a suitable metal catalyst, or a combination of two different suitable metal catalysts (such as bis(triphenylphosphine)palladium(II) chloride and copper (I) iodide and the like), with at least one equivalent of base, inorganic or organic (such as triethylamine and the like), in an organic solvent (such as acetonitrile and the like), undergoing Sonogashira coupling to afford Compound A3. The reaction may be carried out under ambient or elevated temperatures. Optionally, the reaction may also be carried out using microwave radiation at an elevated temperature. Compound A3 is reacted with a Lewis acid (such as trifluoroacetic acid or p-toluenesulfonic acid and the like), with or without an organic solvent (such as toluene or ethyl alcohol and the like), at ambient or elevated temperature, undergoing cyclization to provide Compound A4.

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Scheme B

Compounds of Formula (I), wherein R_2 is a monocyclic or bicyclic aryl, heterocyclyl or heteroaryl ring system, may also be prepared as described in Scheme B below.

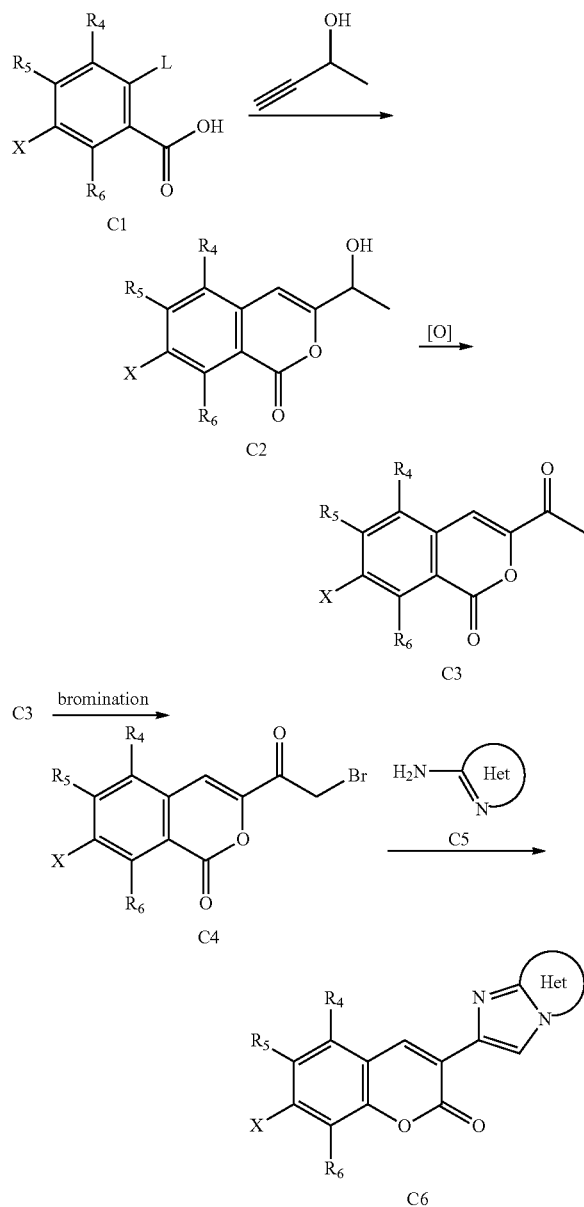


Compound A1 is reacted with trimethylsilylacetylene in the presence of a suitable metal catalyst, or a combination of two different suitable metal catalysts (such as bis(triphenylphosphine)palladium(II) chloride and copper (I) iodide and the like), with at least one equivalent of base, inorganic or organic (such as triethylamine and the like), in an organic solvent (such as acetonitrile and the like), undergoing Sonogashira coupling. The reaction may be carried out at ambient or elevated temperatures. Optionally, the reaction may also be carried out using microwave radiation at an elevated temperature. The resulting trimethylsilylacetylene intermediate is treated with an inorganic base (such as potassium carbonate and the like) in methanol to provide Compound B1. Compound B1 is reacted with Compound B2 using the conditions described in Scheme A, undergoing Sonogashira coupling to provide Compound A3, which may then be converted to Compound A4 by treating with acid as described in Scheme A.

Scheme C

Compounds of Formula (I), wherein R_2 is a monocyclic or bicyclic heterocyclyl or heteroaryl ring system, may be prepared as described in Scheme C below.

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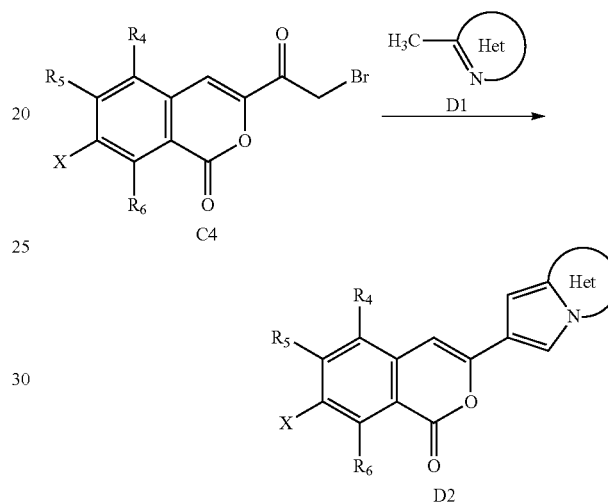


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The α -methyl group of Compound C3 is reacted with an appropriate, selective brominating reagent (such as Br_2 or NBS and the like) to afford Compound C4. Compound C4 is reacted with Compound C5 (wherein the term "Het" refers to an optionally substituted heterocyclyl or heteroaryl ring system containing an amidine-like moiety such as, but not limited to, 2-aminopyridine, 2-aminopyrimidine, 2-aminopyrazine, 3-aminopyridazine, 2-aminothiazole, 4-aminothiazole, 4-aminopyrimidine and the like) to provide Compound C6.

Scheme

Compounds of Formula (I), wherein R_2 is a monocyclic or bicyclic heterocyclyl or heteroaryl ring system, may be prepared as described in Scheme D below.



SPECIFIC SYNTHETIC EXAMPLES

To describe in more detail and assist in understanding, the following non-limiting examples are offered to more fully illustrate the scope of compounds described herein and are not to be construed as specifically limiting the scope thereof. Such variations of the compounds described herein that may be now known or later developed, which would be within the purview of one skilled in the art to ascertain, are considered to fall within the scope of the compounds as described herein and hereinafter claimed. These examples illustrate the preparation of certain compounds. Those of skill in the art will understand that the techniques described in these examples represent techniques, as described by those of ordinary skill in the art, that function well in synthetic practice, and as such

constitute preferred modes for the practice thereof. However, it should be appreciated that those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific methods that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the present description.

Other than in the following examples of the embodied compounds, unless indicated to the contrary, all numbers expressing quantities of ingredients, reaction conditions, experimental data, and so forth used in the specification and claims are to be understood as being modified by the term “about”. Accordingly, all such numbers represent approximations that may vary depending upon the desired properties sought to be obtained by a reaction or as a result of variable experimental conditions. Therefore, within an expected range of experimental reproducibility, the term “about” in the context of the resulting data, refers to a range for data provided that may vary according to a standard deviation from the mean. As well, for experimental results provided, the resulting data may be rounded up or down to present data consistently, without loss of significant figures. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and rounding techniques used by those of skill in the art.

While the numerical ranges and parameters setting forth the broad scope of the present description are approximations, the numerical values set forth in the examples set forth below are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

COMPOUND EXAMPLES

As used above, and throughout the present description, the following abbreviations, unless otherwise indicated, shall be understood to have the following meanings:

Abbreviation	Meaning
Δ	heating (chemistry) or deletion (biology)
AcOH or HOAc	acetic acid
Ac ₂ O	acetic anhydride
Ar	argon
ACN	acetonitrile
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthalene
B(OiPr) ₃	triisopropyl borate
Boc	tert-butoxy-carbonyl
Boc ₂ O	di-tert-butyl dicarbonate
BuOH	n-butanol
° C.	degrees Centigrade
CDI	1,1-carbonyldiimidazole or N,N'-carbonyldiimidazole
(CHO) _n or (HCHO) _n	paraformaldehyde
Cpd	compound
d/h/hr/	day(d)/hour(h, hr or hrs)/
hrs/min/s	minute(min)/second(s)
DavePhos	2-dicyclohexylphosphino-2'-(N,N-dimethylamino)biphenyl
DCE	1,2-dichloroethane
DCM	dichloromethane (CH ₂ Cl ₂)
DIAD	diisopropyl azodicarboxylate
DIEA or DIPEA	N,N-diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyethane
DMF	dimethylformamide
DMSO	dimethylsulfoxide

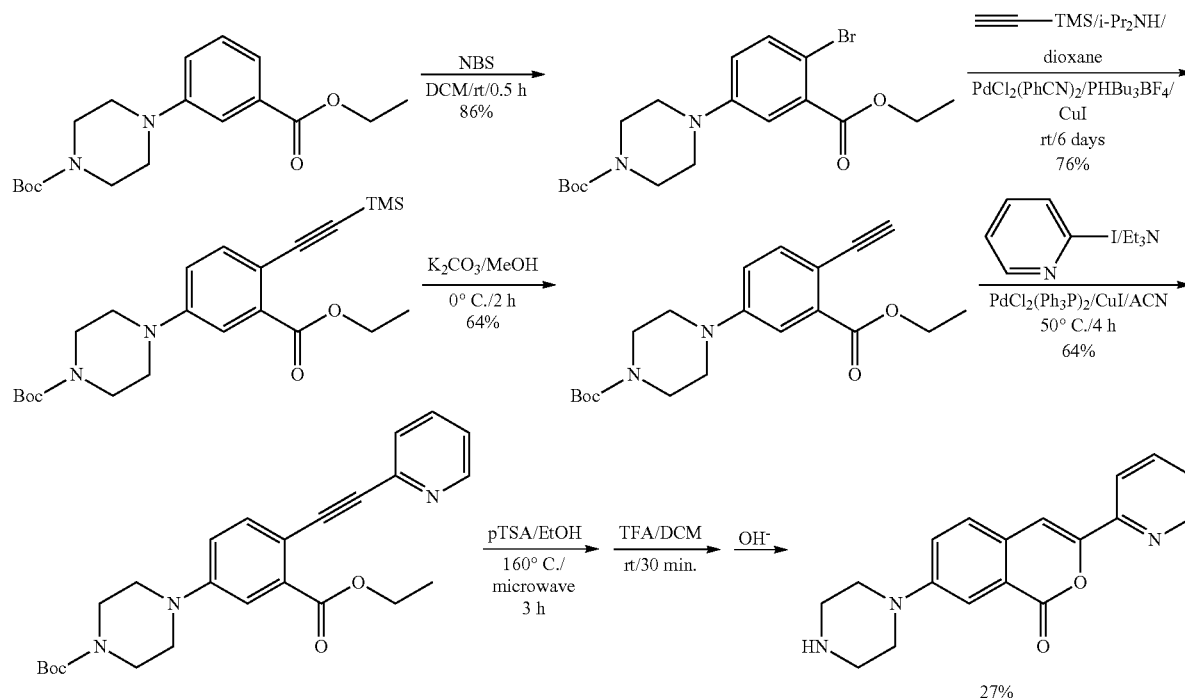
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Abbreviation	Meaning
EDC or EDCI	N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
EtOAc	ethyl acetate
EtOH	ethanol
Et ₂ O	diethyl ether
HCHO	formaldehyde
iPrI	iodopropane
JohnPhos	(2-biphenyl)-di-t-butylphosphine
KOAc	potassium acetate
LAH	lithium aluminum hydride
LC/MS, LCMS or LC-MS	liquid chromatographic mass spectroscopy
LDA	lithium diisopropylamine
LiHMDS or LHMDs	lithium bis(trimethylsilyl)amide
MeOH	methanol
MeI	iodomethane
Me—THF	2-methyltetrahydrofuran
Me ₂ Zn	dimethylzinc
MnO ₂	manganese dioxide
MS	mass spectroscopy
NaH	sodium hydride
NaHS	sodium hydrosulfide
NaHMDS	sodium bis(trimethylsilyl)amide or sodium hexamethyldisilazide
NaI	sodium iodide
NaOAc	sodium acetate
NaOMe	sodium methoxide
NBS	N-bromosuccinimide
NMP	N-methylpyrrolidone
NMR	nuclear magnetic resonance
o/n	overnight
Pd	palladium
Pd/C	palladium on carbon
Pd(dba) ₂	bis(dibenzylideneacetone)palladium
Pd ₂ (dba) ₃ or Pd ₂ dba ₃	tris(dibenzylideneacetone)dipalladium(0)
PdCl ₂ (PhCN) ₂	trans-bis(benzonitrile)di-chloropalladium(II)
PdCl ₂ (dppf), PdCl ₂ dppf or Pd(dppf)Cl ₂	[1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II)
Pd(OAc) ₂	palladium(II) acetate
Pd(PPh ₃) ₄ or Pd(Ph ₃ P) ₄	tetrakis(triphenylphosphine)palladium(0)
Pd(PPh ₃) ₂ Cl ₂ , PdCl ₂ (PPh ₃) ₂ or PdCl ₂ (Ph ₃ P) ₂	bis(triphenylphosphine)palladium(II) dichloride
PHBu ₃ BF ₄ or tBu ₃ PHBF ₄	tri-tert-butylphosphonium tetrafluoroborate
PhI	iodobenzene
PhI(OTFA) ₂	[bis(trifluoroacetoxy)iodo]benzene
PhMe	toluene
POCl ₃	phosphoryl chloride
PPh ₃	triphenylphosphine
PPA	polyphosphoric acid
PPTs	pyridinium p-toluenesulfonate
psi	pounds per square inch pressure
PyBOP	(benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate
rt	room temperature
S-Phos, SPhos or Sphos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
T ₃ P	propylphosphonic anhydride
TEA, Et ₃ N or NEt ₃	triethylamine
TF ₂ O	triflic anhydride
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilane
TMSCl	trimethylchlorosilane or trimethylsilyl chloride
TMSOK	potassium trimethylsilanolate
t-Bu	tert-butyl
TsOH, p-TsOH or pTSA	tosylic acid or p-toluenesulfonic acid
xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

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Example 1

Preparation of Cpd 1



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LCMS revealed a complete conversion was achieved within 2 hours. Then a saturated solution of NH_4Cl (20 mL) and ethyl acetate (200 mL) was added and the organic layer was sepa-

Step A: To a solution of commercially available tert-butyl 4-(3-(ethoxycarbonyl)-phenyl)piperazine-1-carboxylate (13.4 g, 40.0 mmol) in dichloromethane (200 mL), at room temperature while gently stirring, was added N-bromosuccinimide (8.5 g, 48 mmol) portion wise. After the addition, the mixture was stirred at room temperature for 0.5 hours. LCMS analysis of an aliquot revealed a complete disappearance of the starting material. The mixture was treated with a saturated Na_2CO_3 solution (100 mL) and stirred for 15 minutes. The organic layer was separated and dried over Na_2SO_4 . After removal of the solvent, the residue was chromatographed on a silica gel column using a gradient of 0-50% ethyl acetate in hexanes. The bromide intermediate was obtained as a colorless oil (14.2 g, 86%). MS m/z 412.4 $[\text{M}+\text{H}]^+$, 414.4 $[\text{M}+2\text{H}]^+$.

Step B: A 250 mL round bottom flask was charged $\text{PdCl}_2(\text{PhCN})_2$ (0.51 g, 1.33 mmol), PBU_3HBF_4 (0.77 g, 2.66 mmol) and CuI (0.20 g, 1.06 mmol), and purged with argon three times, followed by the addition of dioxane (30 mL) and diisopropylamine (5.6 mL, 4.03 g, 40.0 mmol). The mixture was stirred for 30 minutes, at room temperature, followed by the addition of the bromide intermediate (11.0 g, 26.6 mmol) obtained in Step A and TMS-alkyne (4.51 mL, 3.13 g, 32.0 mmol). The reaction was then stirred at room temperature for 6 days. LCMS analysis of an aliquot showed >95% conversion was achieved. The precipitate was removed by filtration and washed with ethyl acetate. The filtrates were combined and the volatiles were removed on a rotovap. The residue was chromatographed (silica gel, 0-70% ethyl acetate in hexanes) to provide the TMS alkyne intermediate as a brown oil (8.73 g, 76%). MS m/z 431.4 $[\text{M}+\text{H}]^+$.

Step C: A mixture of the TMS alkyne intermediate obtained in Step B (3.10 g, 7.21 mmol), K_2CO_3 (1.49 g, 10.8 mmol) and MeOH (40 mL) was stirred in an ice-water bath.

The aqueous layer was extracted with ethyl acetate (3×30 mL) and the combined organics were evaporated to dryness on a rotovap. The residue was chromatographed (silica gel, 0-10% ethyl acetate in hexanes) to give the alkyne intermediate as a brown oil (1.65 g, 64%). MS m/z 359.3 $[\text{M}+\text{H}]^+$; ^1H NMR (500 MHz, CDCl_3 -d) δ ppm 7.51 (1H, d, $J=8.51$ Hz), 7.44 (1H, d, $J=2.84$ Hz), 6.97-7.03 (1H, m), 4.41 (2H, q, $J=7.15$ Hz), 3.57-3.64 (4H, m), 3.21-3.27 (5H, m), 1.49 (9H, s), 1.41 (3H, t, $J=7.09$ Hz).

Step D: To a mixture of alkyne intermediate obtained in step C (1.10 g, 3.1 mmol), 2-iodopyridine (0.69 g, 3.38 mmol), $\text{PdCl}_2(\text{Ph}_3\text{P})_2$ (0.11 g, 0.15 mmol) and CuI (0.03 g, 0.15 mmol), under argon, was added acetonitrile (6.0 mL) and triethylamine (0.62 g, 6.14 mmol). The mixture was stirred at 50° C. for 4 hours. The volatiles were removed on a rotovap and the residue was chromatographed (silica gel, ethyl acetate in hexanes 0-70%) to give coupling product, tert-butyl 4-(3-(ethoxycarbonyl)-4-(pyridin-2-ylethynyl)phenyl)piperazine-1-carboxylate as a brown oil (1.2 g, 87%). MS m/z 436.4 $[\text{M}+\text{H}]^+$.

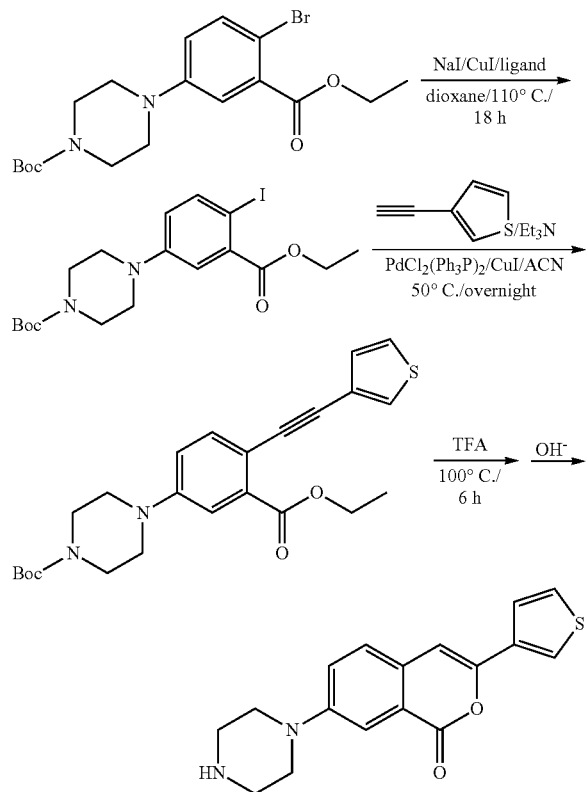
Step E: A mixture of the compound obtained in Step D (0.59 g, 1.4 mmol) and p-toluenesulfonic acid (0.05 g, 0.27 mmol) in ethanol (6.0 mL) was irradiated in a microwave reactor at 180° C. for 3 hours. The mixture was then diluted with water (20 mL) and made basic to pH 8 using Na_2CO_3 . The precipitate was collected, washed with water and dried. The solid was dissolved in dichloromethane (10 mL), treated with TFA (2 mL) for 0.5 h at room temperature, then made basic using Na_2CO_3 to pH 8-9. The dichloromethane layer was separated and the aqueous layer was extracted with additional dichloromethane (3×5 mL). The combined dichloromethane solution was dried over Na_2SO_4 and chromato-

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graphed (silica gel, MeOH in dichloromethane, 0-30%) to give the title compound as a yellow solid (0.071 g, 17%). Melting point 179-181° C.; MS m/z 308.2 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.64-8.69 (1H, m), 7.95 (1H, td, J=7.80, 1.73 Hz), 7.85 (1H, dt, J=7.96, 1.06 Hz), 7.68-7.74 (2H, m), 7.57 (1H, dd, J=8.83, 2.84 Hz), 7.51 (1H, d, J=2.52 Hz), 7.42 (1H, ddd, J=7.57, 4.73, 1.26 Hz), 3.21-3.26 (4H, m), 2.84-2.89 (4H, m).

Example 2

Preparation of Cpd 2



Step A: A mixture of tert-butyl 4-(4-bromo-3-(ethoxycarbonyl)phenyl)piperazine-1-carboxylate, prepared as depicted in Example 1, Step A (22.63 g, 54.8 mmol), CuI (0.52 g, 2.75 mmol), NaI (16.44 g, 109.6 mmol) and N1,N2-dimethylcyclohexane-1,2-diamine (0.87 mL, 5.5 mmol) in dioxane (100 mL) was stirred at 110° C. under argon for 18 hours. The solid was removed by filtration and the filtrate was concentrated to dryness on a rotovap. The residue was chromatographed (silica gel, ethyl acetate in hexanes, 0-30%) to provide the iodide intermediate as a brown oil (26.2 g, 102%). MS m/z 461.2 [M+H]⁺.

Step B: Tert-butyl 4-(3-(ethoxycarbonyl)-4-(thiophen-3-ylethynyl)phenyl)piperazine-1-carboxylate was prepared by employing the Sonagashira coupling procedure depicted in Example 1, Step D from the iodide prepared in Step A above and 3-ethynylthiophene (87%). MS m/z 441.2 [M+H]⁺.

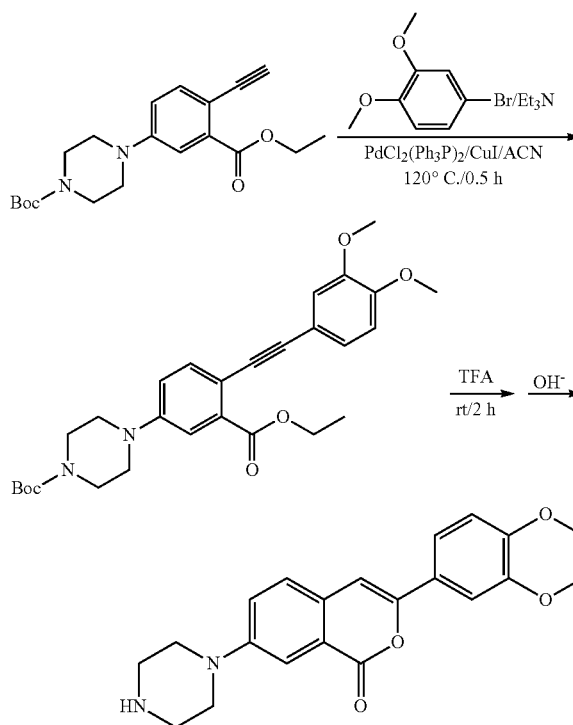
Step C: The compound prepared in Step B (192 mg, 0.44 mmol) was stirred with TFA (1.0 mL) at 100° C. for 6 hours. After cooling, the mixture was diluted with water (5 mL) and neutralized with NaHCO₃. The precipitate was collected and

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washed with water, dichloromethane and acetone to provide the title compound as a brown powder (47 mg, 34%). Melting point 240° C. (decomp.); MS m/z 313.2 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 7.95 (1H, dd, J=2.99, 1.42 Hz), 7.71 (1H, dd, J=5.04, 2.84 Hz), 7.54-7.65 (4H, m), 7.27 (1H, s), 3.45-3.53 (4H, m), 3.20-3.28 (4H, m).

Example 3

Preparation of Cpd 3



Step A: A mixture of tert-butyl 4-(3-(ethoxycarbonyl)-4-ethynylphenyl)piperazine-1-carboxylate, prepared using the chemistry depicted in Example 1, Step C (3.58 g, 10.0 mmol), 3,4-dimethoxybromobenzene (2.60 g, 12.0 mmol), CuI (0.095 g, 0.5 mmol), PdCl₂(Ph₃P)₂ (0.35 g, 0.5 mmol), Et₃N (2.8 mL, 2.02 g, 20.0 mmol) and acetonitrile (10.0 mL) was irradiated in a microwave reactor under argon at 120° C. for 0.5 hours. The mixture was then cooled and chromatographed (silica gel, ethyl acetate in hexanes, 0-70%) to give the alkyne intermediate as a brown oil, which was then chromatographed again (silica gel, ethyl acetate in dichloromethane, 0-10%) to give a colorless oil, homogenous in LCMS analysis. MS m/z 495.2 [M+H]⁺.

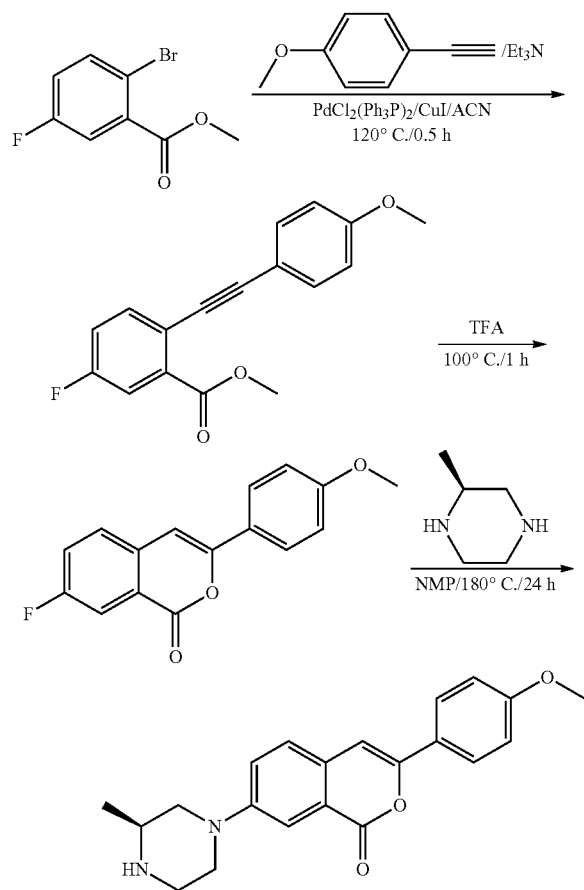
Step B: The alkyne intermediate obtained in Step A, was treated with trifluoroacetic acid (10.0 mL) at room temperature for 1 hour. The solution was diluted with water (50 mL) and neutralized with NaHCO₃ to pH 8-9. The yellow precipitate was collected and washed with water and dried to provide the title compound (1.26 g, 34% overall, 2 steps). Melting point: 142-143° C.; MS m/z 367.2 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 7.52-7.59 (2H, m), 7.46 (1H, d, J=1.58 Hz), 7.42 (1H, dd, J=8.51, 2.21 Hz), 7.38 (1H, d,

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J=2.21 Hz), 7.32 (1H, s), 7.08 (1H, d, J=8.83 Hz), 3.86 (3H, s), 3.81 (3H, s), 3.15-3.22 (4H, m), 2.82-2.89 (4H, m).

Example 4

Preparation of Cpd 31



Step A: A mixture of methyl 2-bromo-5-fluorobenzoate (699 mg, 3.0 mmol), 1-ethynyl-4-methoxybenzene (475 mg, 3.6 mmol), CuI (28.5 mg, 0.15 mmol), PdCl₂(Ph₃P)₂ (105 mg, 0.15 mmol), Et₃N (0.83 mL, 606 mg, 6.0 mmol) and acetonitrile (3.0 mL) was irradiated in a microwave reactor, under argon at 120° C. for 0.5 hours. The mixture was then cooled and chromatographed (silica gel, ethyl acetate in hexanes, 0-10%) to give the alkyne intermediate as a brown oil, used directly in the next step. MS m/z 285.1 [M+H]⁺.

Step B: The alkyne intermediate obtained in Step A was treated with trifluoroacetic acid (6.0 mL) at 100° C. for 1 hour. The volatiles were removed under vacuum and the residue was treated with water and neutralized with NaHCO₃. The precipitate was collected and chromatographed (silica gel, dichloromethane) to give the isocoumarin intermediate (581 mg, 72%, 2 steps). MS m/z 271.1 [M+H]⁺.

Step C: A mixture of the compound obtained in Step B (108 mg, 0.4 mmol) and (S)-2-methylpiperazine (120 mg, 1.2 mmol) in NMP (1.0 mL) was stirred at 180° C. for 24 hours. After cooling, the mixture was loaded onto a silica gel column and chromatographed (MeOH in dichloromethane, 0-20%) to provide the title compound as a yellow powder (24 mg, 17%).

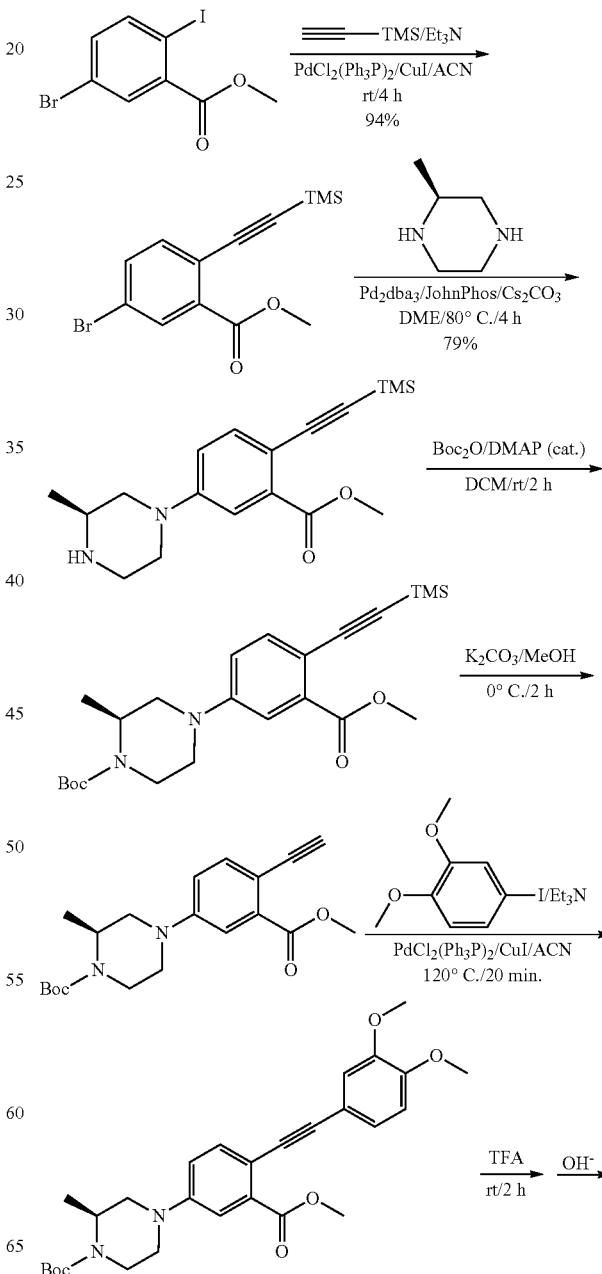
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Melting point: 129-131° C.; MS m/z 351.3 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆) δ ppm 7.80 (2H, d, J=9.14 Hz), 7.51-7.59 (2H, m), 7.46 (1H, d, J=2.21 Hz), 7.26 (1H, s), 7.06 (2H, d, J=8.83 Hz), 3.82 (3H, s), 3.64-3.73 (2H, m), 2.97-3.03 (1H, m), 2.76-2.85 (2H, m), 2.61-2.70 (1H, m), 2.31 (1H, t, J=11.03 Hz), 1.06 (3H, d, J=6.31 Hz).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 4 by substituting the appropriate starting materials, reagents and reaction conditions.

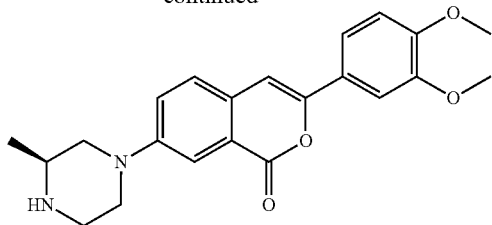
Example 5

Preparation of Cpd 30



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-continued



Step A: A mixture of methyl 5-bromo-2-iodobenzoate (18.4 g, 54.0 mmol), TMS acetylene (8.5 mL, 5.88 g, 60.0 mmol), CuI (0.51 g, 2.7 mmol), PdCl₂(Ph₃P)₂ (1.9 g, 2.7 mmol), Et₃N (15.0 mL, 10.9 g, 108.0 mmol) and acetonitrile (100 mL) was stirred under argon at room temperature for 4 hours. After the removal of the volatiles in vacuo, the residue was chromatographed (silica gel, ethyl acetate in hexanes, 0-20%) to provide the TMS alkyne intermediate as colorless oil (15.7 g, 94%).

Step B: The alkyne intermediate obtained in Step A (9.33 g, 30.0 mmol) was mixed with (S)-2-methylpiperazine (3.60 g, 36.0 mmol), Pd₂dba₃ (0.27 g, 0.6 mmol), JohnPhos (0.18 g, 0.6 mmol) and Cs₂CO₃ (13.7 g, 42.0 mmol). The reaction system was purged with argon three times and the solvent DME (60 mL) was added. The suspension was then stirred at 80° C. for 4 hours. LCMS analysis of an aliquot of the reaction mixture revealed a complete consumption of the starting bromide. Solvent was removed on a rotovap and the residue was chromatographed (silica gel, ethyl acetate in dichloromethane 0-50%) to give (S)-methyl 5-(3-methylpiperazin-1-yl)-2-((trimethylsilyl)ethynyl)benzoate as a brown oil (7.56 g, 79%). MS m/z 331.1 [M+H]⁺.

Step C: The compound obtained in Step B (7.56 g, 22.9 mmol) was treated with di-tert-butyl dicarbonate (7.5 g, 34.4 mmol) and a few crystals of DMAP in dichloromethane (100 mL). After stirring for 2 h at room temperature, LCMS analysis of an aliquot of the reaction mixture revealed a complete disappearance of the starting material. Solvent was removed on a rotovap and the residue was chromatographed (silica gel, ethyl acetate in dichloromethane, 0-10%) to provide (S)-tert-butyl 4-(3-(methoxycarbonyl)-4-((trimethylsilyl)ethynyl)phenyl)-2-methylpiperazine-1-carboxylate as colorless oil (6.49 g, 66%). MS m/z 431.1 [M+H]⁺.

Step D: The compound obtained in Step C (6.49 g, 15.1 mmol) was treated with K₂CO₃ (potassium carbonate) (3.12 g, 22.6 mmol) in MeOH (40 mL) on an ice-water bath for 2 hours. The volatiles were removed on a rotovap and the residue was treated with saturated NH₄Cl (100 mL) and extracted with ethyl acetate (3×150 mL). The combined extracts were then concentrated to dryness and chromatographed (silica gel, ethyl acetate in hexanes 0-80%) to give (S)-tert-butyl 4-(4-ethynyl-3-(methoxycarbonyl)phenyl)-2-methylpiperazine-1-carboxylate as a light brown oil (5.39 g, 100%). MS m/z 359.3 [M+H]⁺.

Step E: A mixture of the intermediate prepared in Step D, (S)-tert-butyl 4-(4-ethynyl-3-(methoxycarbonyl)phenyl)-2-methylpiperazine-1-carboxylate (716 mg, 2.0 mmol), 4-iodo-1,2-dimethoxybenzene (634 mg, 2.4 mmol), CuI (19.0 mg, 0.1 mmol), PdCl₂(Ph₃P)₂ (70 mg, 0.1 mmol), Et₃N (0.56 mL, 202 mg, 4.0 mmol) and acetonitrile (2.0 mL) was irradiated in a microwave reactor, under argon at 120° C. for 20 minutes. The mixture was then cooled and chromatographed twice (silica gel, ethyl acetate in hexanes, 0-50%, then ethyl acetate in dichloromethane, 7.5%) to give the alkyne intermediate as a yellow oil (367 mg, 37%). MS m/z 495.3 [M+H]⁺.

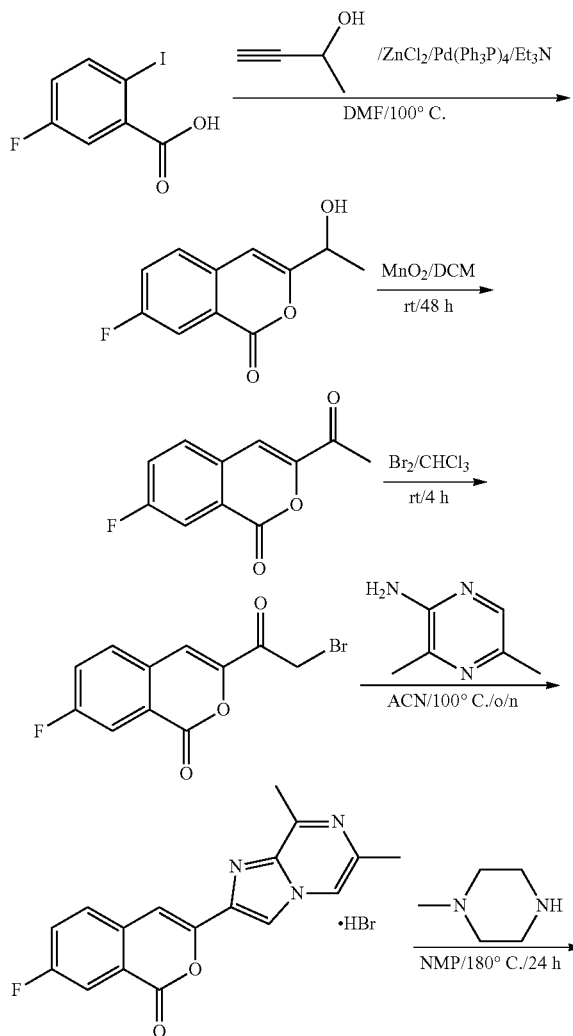
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Step F: The compound obtained in Step E (367 mg, 0.74 mmol) was treated with TFA (5.0 mL) at room temperature for 2 hours. LCMS analysis of an aliquot of the reaction mixture showed a complete conversion achieved. This was diluted with water (40 mL), neutralized with NaHCO₃ and extracted with dichloromethane (3×20 mL). The combined extracts were dried and evaporated to dryness. The yellow residue was triturated with ethyl ether and dried. The title compound was obtained as a yellow powder (228 mg, 81%). Melting point: 155-157° C.; MS m/z 381.5 [M+H]⁺; ¹H NMR (500 MHz, DMSO-d₆): 6 ppm 7.51-7.58 (2H, m), 7.46 (1H, d, J=2.52 Hz), 7.42 (1H, dd, J=8.51, 1.89 Hz), 7.38 (1H, d, J=1.89 Hz), 7.32 (1H, s), 7.07 (1H, d, J=8.83 Hz), 3.86 (3H, s), 3.81 (3H, s), 3.64-3.71 (2H, m), 2.96-3.02 (1H, m), 2.75-2.84 (2H, m), 2.65 (1H, m, J=3.15 Hz), 2.30 (1H, dd, J=11.35, 10.40 Hz), 1.05 (3H, d, J=6.31 Hz).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 5 by substituting the appropriate starting materials, reagents and reaction conditions.

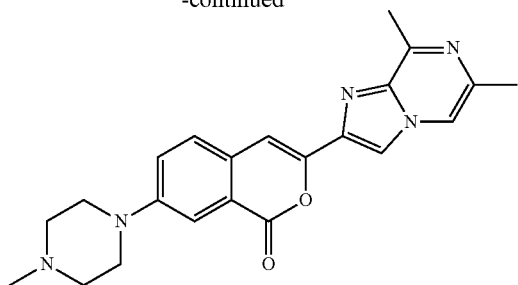
Example 6

Preparation of Cpd 65



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-continued



Step A: A mixture of 5-fluoro-2-iodobenzoic acid (9.04 g, 34.0 mmol), but-3-yn-2-ol (5.7 mL, 5.46 g, 78.0 mmol), ZnCl_2 (4.62 g, 34.0 mmol), $\text{Pd}(\text{Ph}_3\text{P})_4$ (1.96 g, 1.7 mmol), Et_3N (14.2 mL, 10.3 g, 102.0 mmol) and DMF (50 mL) was stirred under argon at 100°C . for 2 hours. After the removal of the volatiles under vacuum, the residue was chromatographed (silica gel, ethyl acetate in hexanes, 0-50%) to provide intermediate 7-fluoro-3-(1-hydroxyethyl)-1H-isochromen-1-one as a brown oil (5.93 g, 84%). MS m/z 209.2 $[\text{M}+\text{H}]^+$; ^1H NMR (500 MHz, CDCl_3 -d): 6 ppm 7.91-7.96 (1H, m), 7.43-7.47 (2H, m), 6.55-6.59 (1H, m), 4.67 (1H, q, $J=6.52$ Hz), 1.57 (3H, d, $J=6.62$ Hz).

Step B: The intermediate obtained in Step A (5.93 g, 28.5 mmol) was dissolved in dichloromethane (50 mL) and treated with MnO_2 (24.8 g, 285 mmol) for 48 h at room temperature. The solvent was removed on a rotovap and the residue was suspended in dichloromethane (500 mL) and stirred for 0.5 hours. The mixture was filtered and the solid was thoroughly washed with dichloromethane (4 \times 100 mL). The combined filtrates were evaporated to dryness on a rotovap and chromatographed (silica gel, ethyl acetate in dichloromethane 0-20%) to provide the ketone intermediate as white needles (3.88 g, 66%). MS m/z 207.1 $[\text{M}+\text{H}]^+$; ^1H NMR (500 MHz, CDCl_3 -d): 6 ppm 8.01-8.06 (1H, m), 7.68 (1H, dd, $J=8.51$, 5.04 Hz), 7.51-7.57 (1H, m), 7.40 (1H, d, $J=0.63$ Hz), 2.59 (3H, s)

Step C: The ketone intermediate obtained in Step B (2.63 g, 12.8 mmol) was dissolved in chloroform (30 mL) and treated with bromine (0.72 mL, 2.25 g, 14.0 mmol). The mixture was stirred at room temperature for 1 hour, followed by the addition of hexanes (150 mL) and the mixture was stirred for 15 minutes. The precipitate was collected by filtration and washed with hexanes, water and dried. The filtrate was washed with NaHCO_3 and concentrated. The residue was chromatographed (silica gel, ethyl acetate in dichloromethane, 0-5%) to provide additional bromoketone intermediate (total 3.48 g, 96%). MS m/z 283.0 $[\text{M}-\text{H}]^-$, 285.0 $[\text{M}-\text{H}]^-$; ^1H NMR (500 MHz, CDCl_3 -d): 6 ppm 8.05 (1H, dd, $J=8.20$, 2.84 Hz), 7.72 (1H, dd, $J=8.51$, 5.04 Hz), 7.54-7.60 (1H, m), 7.52 (1H, s), 4.47 (2H, s).

Step D: The bromoketone intermediate obtained in Step C (1.43 g, 5.0 mmol) was mixed with 3,5-dimethylpyrazin-2-amine (0.67 g, 5.5 mmol) and acetonitrile (10.0 mL) in a sealed tube. The mixture was stirred at 100°C . overnight and cooled to room temperature. Ethyl acetate (20 mL) was added and the precipitate was collected, washed with ethyl acetate and then dried, providing 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-fluoro-1H-isochromen-1-one hydrobromide (1.81 g, 93%). MS m/z 310.3 $[\text{M}+\text{H}]^+$.

Step E: A mixture of the compound obtained in Step D (390 mg, 1.0 mmol), N-methylpiperazine (300 mg, 3.0 mmol) in NMP (2.0 mL) was stirred at 180°C . for 24 h under argon.

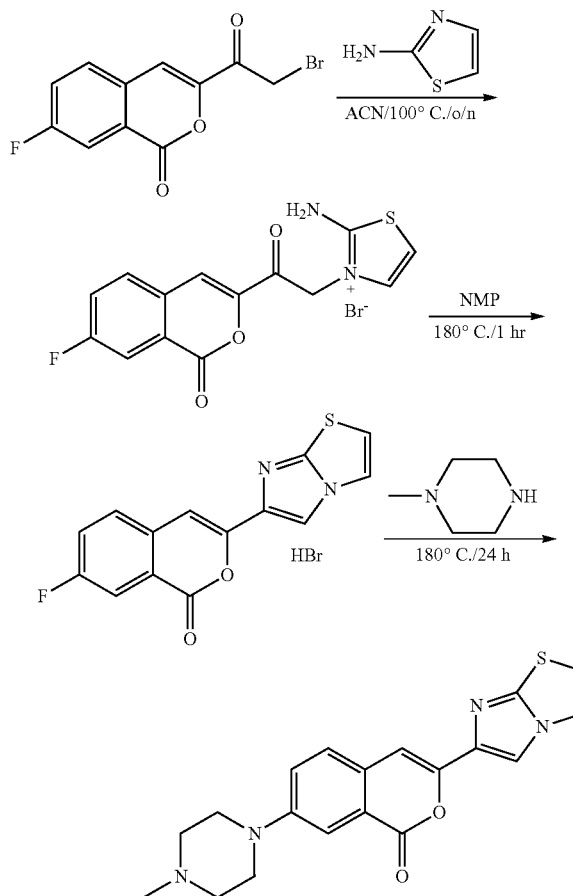
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After cooling to room temperature, the mixture was loaded on a silica gel column. Flash chromatography (silica gel) was performed using MeOH in dichloromethane (0-30%) to provide the title compound as a yellow powder (223 mg, 57%). Melting point: $240-241^\circ\text{C}$.; MS m/z 390.1 $[\text{M}+\text{H}]^+$; ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ ppm 8.34 (1H, s), 8.24-8.27 (1H, m), 7.70 (1H, d, $J=8.83$ Hz), 7.57 (1H, dd, $J=8.83$, 2.52 Hz), 7.50 (1H, d, $J=2.52$ Hz), 7.42 (1H, s), 3.26-3.31 (4H, m), 2.74 (3H, s), 2.45-2.49 (4H, m), 2.38 (3H, d, $J=0.95$ Hz), 2.24 (3H, s).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 6 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 7

Preparation of Cpd 33



Step A: The bromoketone intermediate obtained in Step C, Example 6 (285 mg, 1.0 mmol) was mixed with 2-aminothiazole (110 mg, 1.1 mmol) and acetonitrile (4.0 mL) in a sealed tube. The mixture was stirred at 100°C . for 0.5 hr and cooled to room temperature. Ethyl acetate (10 mL) was added and the precipitate was collected by filtration. The precipitate was washed with ethyl acetate and dried to provide the intermediate, 2-amino-3-(2-(7-fluoro-1-oxo-1H-isochromen-3-yl)-2-oxoethyl)thiazol-3-ium bromide (355 mg, 92%) homogenous in LCMS analysis. MS m/z 305.0 M^+ .

Step B: A mixture of the compound obtained in Step A (193 mg, 0.5 mmol) in NMP (1.0 mL) was stirred at 180°C . for 1

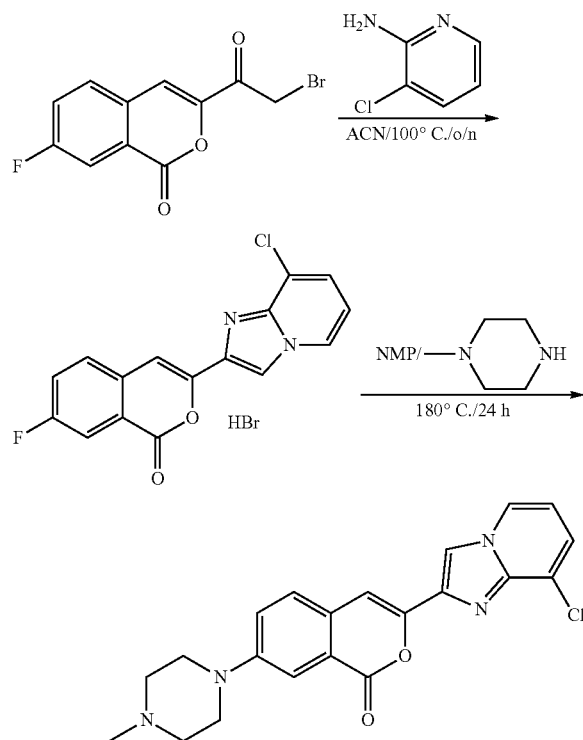
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hr until LCMS analysis of an aliquot of the reaction mixture showed all the starting material had been converted to the cyclization intermediate. MS m/z 287.0 $[M+H]^+$. The mixture was cooled to room temperature and N-methylpiperazine (150 mg, 1.5 mmol) was added under argon. The resulting mixture was then stirred at 180° C. for 24 hours. After cooling, the mixture was loaded on a silica gel column and the title compound was eluted with MeOH in dichloromethane (0-30%) as a yellow powder (32 mg, 17%). Melting point: 236-238° C.; MS m/z 367.2 $[M+H]^+$; 1H NMR (500 MHz, DMSO- d_6) δ ppm 8.12 (1H, s), 7.93 (1H, d, $J=4.41$ Hz), 7.52-7.63 (2H, m), 7.48 (1H, s), 7.32 (1H, d, $J=4.41$ Hz), 7.17 (1H, s), 3.23-3.30 (4H, m), 2.43-2.49 (4H, m), 2.23 (3H, s).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 7 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 8

Preparation of Cpd 61



Step A: Following the procedure described in Example 6, Step D, the intermediate, 3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-fluoro-1H-isochromen-1-one hydrobromide (244 mg, 62%), was obtained from commercially available 3-chloro-2-aminopyridine. MS m/z 315.1 $[M+H]^+$.

Step B: Following the procedure described in Example 6, Step E, from 3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-fluoro-1H-isochromen-1-one hydrobromide (99 mg, 0.25 mmol) and N-methylpiperazine (86 mg, 0.75 mmol), the title compound, was obtained as a yellow powder (19 mg, 19%).

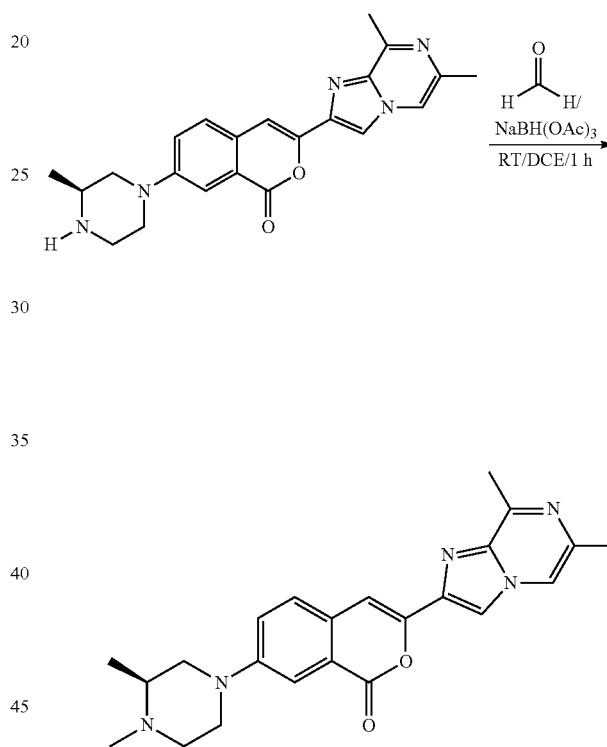
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Melting point: 240° C. (decomposition); MS m/z 395.0 $[M+H]^+$; 1H NMR (500 MHz, DMSO- d_6) δ ppm 8.57 (1H, dd, $J=6.94, 0.95$ Hz), 8.46 (1H, s), 7.72 (1H, d, $J=8.83$ Hz), 7.58 (1H, s), 7.52 (1H, dd, $J=7.25, 0.95$ Hz), 7.51 (1H, d, $J=2.52$ Hz), 7.44 (1H, s), 6.95 (1H, dd, $J=7.41, 6.78$ Hz), 3.28-3.31 (4H, m), 2.47 (4H, m), 2.24 (3H, s).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 8 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 9

Preparation of Cpd 66



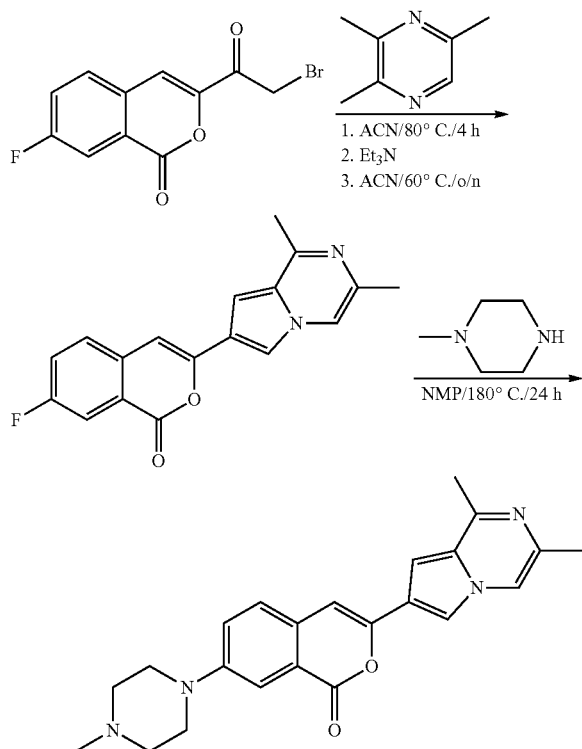
(S)-3-(6,8-Dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(3-methylpiperazin-1-yl)-1H-isochromen-1-one (78 mg, 0.2 mmol), prepared according to the procedure of Example 6, was suspended in dichloroethane (1.0 mL) followed by the addition of formaldehyde (0.4 mL, 37%, 4.9 mmol) and NaBH(OAc)₃ (85 mg, 0.4 mmol). The mixture was stirred at room temperature for 1 hour, then diluted with dichloromethane (5.0 mL) and neutralized with NaHCO₃. The dichloromethane layer was separated and the aqueous layer was extracted with dichloromethane (3x2.0 mL). The extracts were combined and dried over Na₂SO₄ and chromatographed (silica gel, MeOH in dichloromethane 0-20%) to provide the title compound as a pale yellow powder (55 mg, 68%). Melting point: 255-256° C.; MS m/z 404.1 $[M+H]^+$; 1H NMR (500 MHz, DMSO- d_6) δ ppm 8.33 (1H, s), 8.23-8.26 (1H, m), 7.68 (1H, d, $J=8.83$ Hz), 7.56 (1H, dd, $J=8.83, 2.52$ Hz), 7.48 (1H, d, $J=2.52$ Hz), 7.40 (1H, s), 3.65-3.76 (2H, m), 2.82-2.91 (2H, m), 2.73 (3H, s), 2.37 (3H, d, $J=0.95$ Hz), 2.21-2.28 (4H, m), 2.09-2.19 (1H, m), 1.08 (3H, d, $J=6.31$ Hz).

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As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 9 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 10

Preparation of Cpd 76



The bromoketone intermediate obtained in Step C, Example 6 (855 mg, 3.0 mmol) was mixed with 2,3,5-trimethylpyrazine (402 mg, 3.3 mmol) and acetonitrile (10.0 mL) in a sealed tube. The mixture was stirred at 80° C. for 4 hr and cooled to room temperature, followed by the addition of triethylamine (1.3 mL, 9.0 mmol). After stirring for 0.5 hours at room temperature, the mixture was stirred at 60° C. overnight. The solvent was removed on a rotovap and the residue was chromatographed (silica gel, ethylacetate in dichloromethane 30%) to provide the intermediate, 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-fluoro-1H-isochromen-1-one (782 mg, 85%). MS *m/z* 309.3 [M+H]⁺.

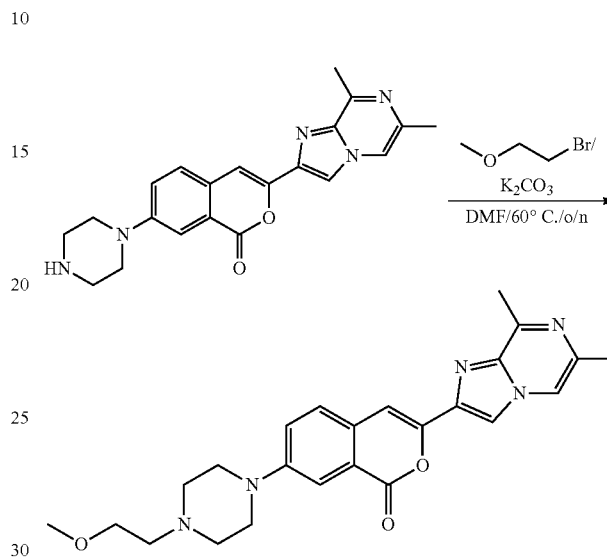
Step B: Following the procedure described in Example 6, Step E, from 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-fluoro-1H-isochromen-1-one (77 mg, 0.25 mmol) and N-methylpiperazine (75 mg, 0.75 mmol), the title compound was obtained as a yellow powder (44 mg, 45%). Melting point: 219-221° C.; MS *m/z* 389.5 [M+H]⁺; ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.03 (1H, d, *J*=1.58 Hz), 7.97 (1H, d, *J*=0.63 Hz), 7.56 (1H, d, *J*=2.84 Hz), 7.47-7.53 (2H, m), 7.22 (1H, s), 7.16-7.19 (1H, m), 3.24-3.30 (4H, m), 2.57 (3H, s), 2.45-2.49 (4H, m), 2.28 (3H, d, *J*=0.95 Hz), 2.24 (3H, s).

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As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 10 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 11

Preparation of Cpd 85

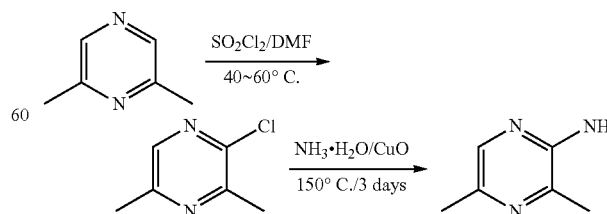


Cpd 65 obtained in Example 6 (188 mg, 0.5 mmol) was mixed with 1-bromo-2-ethoxyethane (104 mg, 0.75 mmol), K₂CO₃ (172 mg, 1.25 mmol) and DMF (1.0 mL) in a sealed tube. The mixture was stirred at 60° C. overnight, cooled to room temperature and chromatographed over silica gel (methanol in dichloromethane, 0-20%) to provide the title compound as a yellow powder (100 mg, 46%). Melting point: 192-194° C.; MS *m/z* 434.3 [M+H]⁺. ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.36 (1H, s), 8.25-8.29 (1H, m), 7.71 (1H, d, *J*=8.83 Hz), 7.57 (1H, dd, *J*=8.83, 2.52 Hz), 7.50 (1H, d, *J*=2.52 Hz), 7.43 (1H, s), 3.48 (2H, t, *J*=5.83 Hz), 3.27-3.31 (4H, m), 3.26 (3H, s), 2.74 (3H, s), 2.56-2.62 (4H, m), 2.54 (2H, t, *J*=5.83 Hz), 2.38 (3H, d, *J*=0.95 Hz).

As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 11 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 12

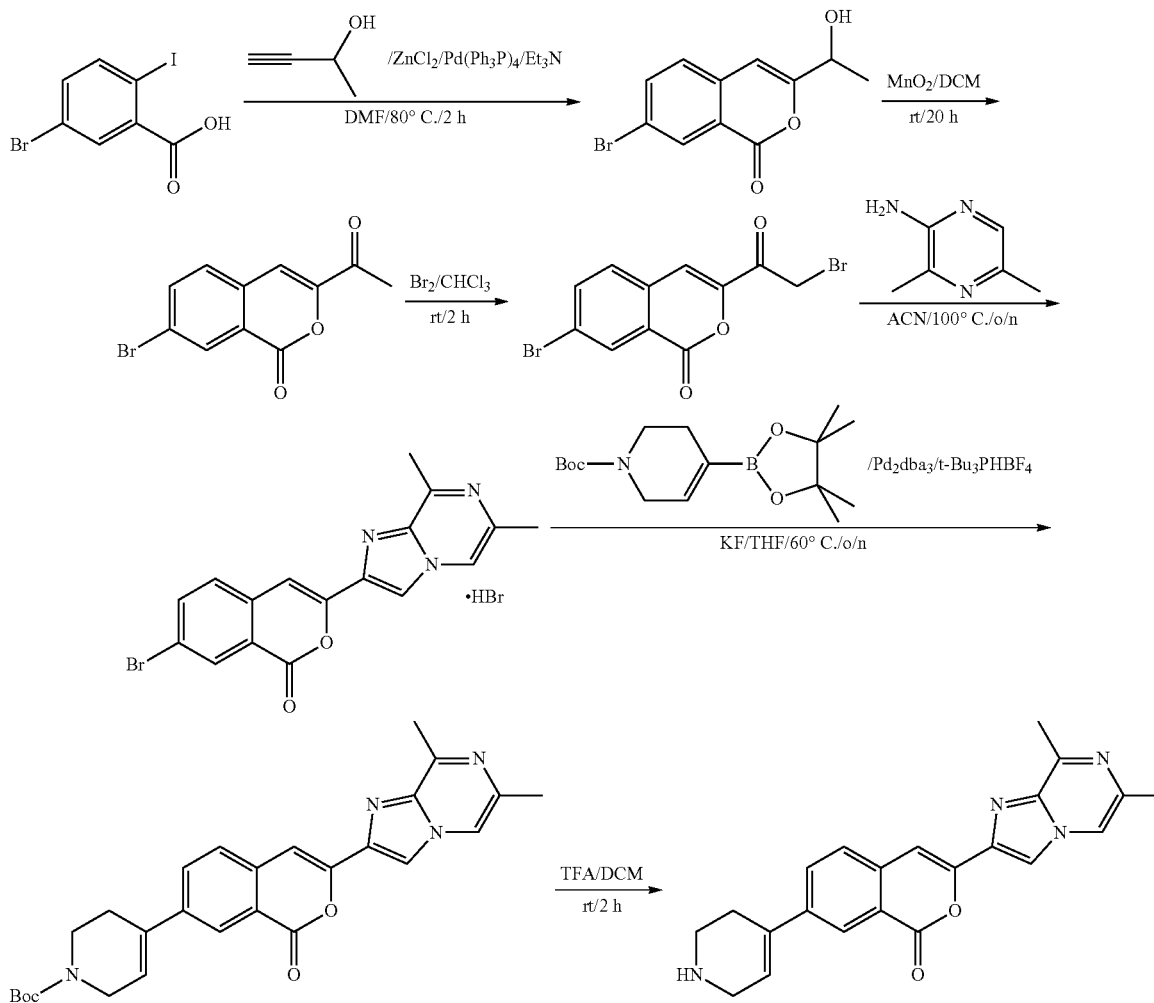
Preparation of Cpd 88



Part 1, Step A: To a cooled solution of 2,6-dimethylpyrazine (108 g, 1.0 mol) in DMF (260 mL) on an ice/H₂O bath,

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while stirring vigorously, was added sulfuryl chloride (270 mL, 3.3 mol). The rate of addition was controlled to maintain the reaction temperature between 40-60° C. for about 2 hours. After the addition, the cooling bath was removed and the mixture was stirred for an additional 0.5 hours. LCMS analysis of an aliquot showed <5% starting material remained. The reaction mixture was then cooled in an icewater bath and, while maintaining the temperature below 35° C., quenched carefully with 10 M NaOH (1 L), followed by the addition of Na₂CO₃ (solid) to pH 6. After the addition of water (800 mL),



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residue was treated with ethyl acetate (500 mL), then stirred for 15 minutes and filtered. The precipitate was washed with additional ethyl acetate (about 1.5 L) until the starting material was no longer detected in the filtrate. The filtrates were combined and concentrated. The residue was chromatographed on a silica gel column (MeOH/CH₂Cl₂, 0-10%) to furnish a yellowbrownish solid (20.7 g, 84%). ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 7.68 (1H, s), 2.46 (3H, s), 2.42 (3H, d, J=0.63 Hz).

the mixture was distilled. The distillate was collected and the organics were separated. The aqueous layer was extracted with ethyl ether (100 mL×3) and the ether extracts were combined with the organics separated previously. The combined extracts were washed with water (30 mL×3), then brine and dried over Na₂SO₄. After the extracts were concentrated, the residue was distilled and the product was collected as a colorless liquid, approximate boiling point: 127° C. at 154 mmHg (99.1 g, 69%). ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.05 (1H, s), 2.61 (3H, d, J=0.63 Hz), 2.50 (3H, s).

Part 1, Step B: A mixture of 2-chloro-3,5-dimethylpyrazine (28.5 g, 0.2 mol), prepared above, CuO (0.8 g, 0.01 mol) and concentrated aqueous NH₃ (28-30%, 150 mL) was stirred in a sealed pressure vessel at 150° C. for 3 days. After cooling, the mixture was concentrated to dryness and the

Part 2, Step A: A mixture of 5-bromo-2-iodobenzoic acid (12.6 g, 38.5 mmol), but-3-yn-2-ol (3.1 mL, 2.96 g, 42.4 mmol), ZnCl₂ (5.2 g, 38.5 mmol), Pd(Ph₃P)₄ (2.23 g, 1.9 mmol), Et₃N (16.0 mL, 11.7 g, 115.5 mmol) and DMF (80 mL) was stirred under argon at 80° C. for 2 hours. After removal of the volatiles under vacuum, the residue was chromatographed (silica gel, ethyl acetate in hexanes, 0-100%) to provide the intermediate 7-bromo-3-(1-hydroxyethyl)-1H-isochromen-1-one as a brown oil (7.5 g, 72%). ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.38-8.45 (1H, m), 7.81 (1H, dd, J=8.35, 2.05 Hz), 7.32 (1H, d, J=8.51 Hz), 6.52-6.59 (1H, m), 4.66 (1H, qd, J=6.52, 0.95 Hz), 1.54-1.60 (3H, m).

Part 2, Step B: The intermediate obtained in Part 2, Step A (7.5 g, 27.7 mmol) was dissolved in dichloromethane (100 mL) and treated with MnO₂ (48.0 g, 554 mmol) for 20 hours

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at room temperature. The solid was filtered and washed thoroughly with dichloromethane (4×200 mL). The combined filtrates were evaporated to dryness on a rotovap and chromatographed (silica gel, ethyl acetate/CH₂Cl₂, 0-20%) to provide the product as white needles (4.5 g, 61%). ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.51 (1H, dd, J=2.84, 0.63 Hz), 7.92 (1H, dd, J=8.35, 2.05 Hz), 7.53 (1H, d, J=8.51 Hz), 7.36 (1H, s), 2.59 (3H, s).

Part 2, Step C: The intermediate obtained in Part 2, Step B (23.0 g, 86.0 mmol) was dissolved in chloroform (400 mL) and treated with bromine (4.64 mL, 14.5 g, 90.3 mmol). The mixture was stirred at room temperature for 2 hours, followed by the addition of hexanes (1000 mL) and the resulting mixture was stirred for 15 minutes. The precipitate was collected by filtration and washed with hexanes, then water and dried. The filtrate was washed with NaHCO₃ and concentrated. The residue was chromatographed (silica gel, ethyl acetate/CH₂Cl₂, 0-5%) to provide additional intermediate (total of 27.0 g, 91%). ¹H NMR (500 MHz, CHLOROFORM-d) δ ppm 8.48-8.56 (1H, m), 7.94 (1H, dd, J=8.35, 2.05 Hz), 7.56 (1H, d, J=8.20 Hz), 7.48 (1H, s), 4.46 (2H, s).

Part 2, Step D: The intermediate obtained in Part 2, Step C (4.84 g, 14.0 mmol) was mixed with 3,5-dimethylpyrazin-2-amine (1.81 g, 14.7 mmol), obtained in Part 1, and acetonitrile (30 mL) in a sealed tube. The mixture was stirred at 100° C. overnight and cooled to room temperature. Ethyl acetate (60 mL) was added and the precipitate was collected, then washed with ethyl acetate and dried, providing 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-bromo-1H-isochromen-1-one hydrobromide (5.04 g, 80%). MS m/z 370.2, 372.2 [M+H]⁺. ¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.50-8.54 (1H, m), 8.34 (1H, s), 8.21 (1H, d, J=1.89 Hz), 8.02 (1H, dd, J=8.35, 2.05 Hz), 7.80 (1H, d, J=8.51 Hz), 7.51-7.57 (1H, m), 2.79 (3H, s), 2.41 (3H, d, J=0.95 Hz).

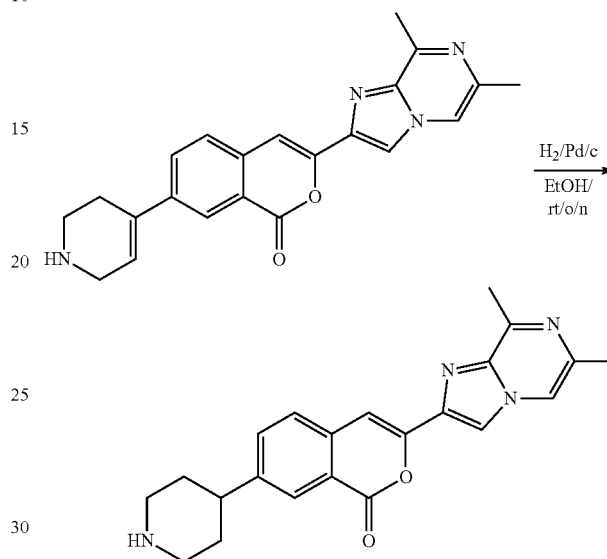
Part 2, Step E: 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-bromo-1H-isochromen-1-one hydrobromide, obtained in Part 2, Step D (1.13 g, 2.5 mmol) was mixed with tert-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5,6-dihydropyridine-1(2H)-carboxylate (1.55 g, 5.0 mmol), Pd₂ dba₃ (0.12 g, 0.013 mmol), KF (0.87 g, 15.0 mmol), t-Bu₃PHBF₄ (0.087 g, 0.3 mmol) and THF (10.0 mL). Under an Argon blanket, the reaction mixture was stirred at 60° C. overnight. After cooling, the mixture was diluted with CH₂Cl₂ (10 mL) and filtered. The precipitate was washed with CH₂Cl₂ and the filtrates were combined and evaporated. The residue was treated with 10% TFA in CH₂Cl₂ (55 mL) for 2 hours at room temperature. The volatiles were removed and the residue was neutralized with NaHCO₃. After being evaporated to dryness, the residue was suspended in 10% MeOH in CH₂Cl₂ and filtered. The filtrate was chromatographed (silica gel, MeOH/CH₂Cl₂, 0-20%) to provide the title compound as a white powder (0.92 g, 99%). Melting point: 266° C. (dec.); MS m/z 373.2 [M+H]⁺. ¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.45 (1H, s), 8.27-8.30 (1H, m), 8.18 (1H, d, J=1.00 Hz), 8.04 (1H, dd, J=1.00 Hz), 7.88 (1H, d, J=8.51 Hz), 7.56 (1H, s), 6.41-6.49 (1H, m), 3.80-3.87 (2H, m), 3.38 (2H, t, J=6.15 Hz), 2.76-2.81 (2H, m), 2.75 (3H, s), 2.39 (3H, d, J=0.95 Hz).

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As shown in Table 1 below, additional compounds disclosed herein may be prepared according to Example 12 by substituting the appropriate starting materials, reagents and reaction conditions.

Example 13

Preparation of Cpd 96



Cpd 88 obtained in Example 12 (37.2 mg, 0.1 mmol) was mixed with 10% Pd/C (8.0 mg) and EtOH (1.0 mL) and hydrogenated at room temperature overnight using a hydrogen balloon. The mixture was diluted with CH₂Cl₂ (2.0 mL) and filtered over Celite. The filtrate was collected and chromatographed (silica gel, 0-20% MeOH in CH₂Cl₂) to provide the title compound as a white powder (21.0 mg, 56%). Melting point: 218-220° C.; MS m/z 375.2 [M+H]⁺. ¹H NMR (500 MHz, DMSO-d₆) δ ppm 8.43 (1H, s), 8.29 (1H, d, J=0.63 Hz), 8.02 (1H, d, J=1.58 Hz), 7.85 (1H, d, J=8.20 Hz), 7.76 (1H, dd, J=8.20, 1.89 Hz), 7.54 (1H, s), 3.38-3.47 (2H, m), 2.96-3.10 (3H, m), 2.76 (3H, s), 2.40 (3H, d, J=0.95 Hz), 1.97-2.08 (2H, m), 1.76-1.92 (2H, m).

Table 1 provides isolated compounds of a free base form of a compound of Formula (I) that may be prepared according to the procedures of the indicated Example by substituting the appropriate starting materials, reagents and reaction conditions. The preparation of any salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer from a free base form of a compound of Formula (I) is also contemplated and further included within the scope of the description herein. Where a free base form of the compound was not isolated from the salt form, a person of ordinary skill in the art could be expected to perform the required reactions to prepare and isolate the free base form of the compound.

The term "Cpd" represents Compound number, the term "Ex" represents "Example Number" (wherein * indicates that the corresponding Example for the Compound is provided above), the term "M.P." represents "Melting Point (° C.)," the term "MS" represents "Mass Spectroscopy Peak(s) m/z M⁺, [M+H]⁺, [M+2H]⁺, [M-H]⁻ or [M-2H]⁻," the term "D" represents "Decomposition/Decomposed," the term "DR" represents "Decomposition Range," the term "S" represents "Softens," the term "ND" indicates that the value was "Not Determined" and the term "NI" indicates that the compound was "Not Isolated."

TABLE 1

Ex	Cpd	Name	M.P.	MS
1*	1	7-(piperazin-1-yl)-3-(pyridin-2-yl)-1H-isochromen-1-one	179-181	308.2
2*	2	7-(piperazin-1-yl)-3-(thiophen-3-yl)-1H-isochromen-1-one	240 (D)	313.2
3*	3	3-(3,4-dimethoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one	142-143	367.2
4	4	7-(4-methylpiperazin-1-yl)-3-(pyridin-2-yl)-1H-isochromen-1-one	174-176	322.3
4	5	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(pyridin-2-yl)-1H-isochromen-1-one	168-170	336.3
4	6	3-(2,2-difluoro-1,3-benzodioxol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	220-222	387.2
4	7	3-(2,2-difluoro-1,3-benzodioxol-5-yl)-7-(4-methyl-1,4-diazepan-1-yl)-1H-isochromen-1-one	175-177	415.3
4	8	3-(1,3-benzothiazol-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	324-326	364.2
4	9	3-(1,3-benzothiazol-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	310-312	392.3
4	10	3-(1,3-benzothiazol-2-yl)-7-(1,4-diazepan-1-yl)-1H-isochromen-1-one	277-279	378.3
4	11	3-(1,3-benzothiazol-2-yl)-7-(4-methyl-1,4-diazepan-1-yl)-1H-isochromen-1-one	270-272	392.3
3	12	3-(1,3-benzodioxol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	267-269	351.2
3	13	3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	253-255	365.2
3	14	3-(3,5-difluorophenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one	200-202	343.1
4	15	3-(1,3-benzodioxol-5-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	297-299	365.2
4	16	3-(3,4-dimethoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	ND	381.3
4	17	3-(3,4-dimethoxyphenyl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	309-311	395.3
4	18	3-(3-methoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one	140-142	337.2
4	19	3-(3-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	140-142	351.2
4	20	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(3-methoxyphenyl)-1H-isochromen-1-one	195-197	365.3
4	21	7-(1,4-diazepan-1-yl)-3-(3-methoxyphenyl)-1H-isochromen-1-one	148-150	351.2
4	22	3-(2-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	118-120	351.3
4	23	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(2-methoxyphenyl)-1H-isochromen-1-one	154-157	365.3
4	24	3-(4-methoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one	235-238	337.2
4	25	3-(4-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	166-168	351.3
8	26	3-(imidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	248 (D)	347.2
8	27	3-(imidazo[1,2-a]pyridin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	252-254	361.3
7	28	3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	224-226	353.3
4	29	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(4-methoxyphenyl)-1H-isochromen-1-one	109-110	365.3
5*	30	3-(3,4-dimethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	155-157	381.3
4*	31	3-(4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	129-131	351.3
5	32	3-(2-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	143-146	351.3
7*	33	3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	236-238	367.2
4	34	7-[(3S)-3-methylpiperazin-1-yl]-3-(pyridin-2-yl)-1H-isochromen-1-one	343-345	322.1
4	35	3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	175-177	379.2
4	36	3-(1,3-benzothiazol-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	236-238	378.2
4	37	3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	175-177	379.2

TABLE 1-continued

Ex	Cpd	Name	M.P.	MS
4	38	3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	163-165	379.2
4	39	3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	218-220	393.3
5	40	3-(1,3-benzodioxol-5-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	248-250	365.3
5	41	3-(3,4-dihydroxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	220-222	353.2
5	42	3-(4-ethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	239-240	365.3
5	43	3-(3-fluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	209-211	369.1
5	44	3-(3-hydroxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	256-258	337.2
5	45	3-(2-fluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	167-168	369.2
5	46	3-(3-chloro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	187-189	385.2
5	47	3-(4-fluoro-3-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	118-120	369.2
5	48	3-(5-fluoro-2-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	115-117	369.2
5	49	3-(3,5-difluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	170-173	387.2
5	50	3-(2,4-dimethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	231 (D)	381.6
3	51	3-(4-ethoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one	245 (D)	351.8
3	52	3-(2-methyl-1-benzofuran-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	206-208	361.7
4	53	3-[3-(difluoromethoxy)phenyl]-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	132-134	387.0
4	54	3-[4-(difluoromethoxy)phenyl]-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	121-123	387.0
7	55	3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	340-342	367.0
6	56	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	225-227	362.0
6	57	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	242-244	376.1
6	58	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	279-281	390.1
8	59	3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	343-345	381.0
8	60	3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	314-316	395.0
8*	61	3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	240 (D)	395.0
6	62	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	>330	376.2
6	63	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	325-327	390.2
6	64	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	244-246	404.3
6*	65	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	240-241	390.1
9*	66	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one	255-256	404.1
9	67	3-(3,4-dimethoxyphenyl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one	138-140	395.1
6	68	7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	255-257	402.5
6	69	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one	282-285	416.5
6	70	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one	282-285	416.5
6	71	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	331-333	390.5
9	72	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one	252-254	404.1
6	73	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one	238-240	404.6
6	74	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	260-262	418.5
6	75	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-hydroxyethyl)piperazin-1-yl]-1H-isochromen-1-one	248-250	420.5

TABLE 1-continued

Ex	Cpd	Name	M.P.	MS
10*	76	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	219-221	389.5
10	77	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one	202-204	403.5
10	78	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	213-215	417.5
10	79	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[4-(2-hydroxyethyl)piperazin-1-yl]-1H-isochromen-1-one	240-242	419.6
10	80	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[(8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one	220-222	415.1
10	81	3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one	218-220	415.1
6	82	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-propylpiperazin-1-yl)-1H-isochromen-1-one, and	244-246	418.2
6	83	7-(4-tert-butylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;	300-302	432.3
9	84	7-(4-cyclopropylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	260 (D)	416.3
11*	85	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-methoxyethyl)piperazin-1-yl]-1H-isochromen-1-one	192-194	434.3
9	86	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-methylpropyl)piperazin-1-yl]-1H-isochromen-1-one	243-245	432.3
9	87	7-(4-cyclobutylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	279-281	430.3
12*	88	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	266 (D)	373.2
9	89	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	240-242	387.3
9	90	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-ethyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	227-229	401.3
9	91	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(propan-2-yl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one	259-261	415.3
11	92	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(2-methoxyethyl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one	174-176	431.2
9	93	7-(1-cyclopropyl-1,2,3,6-tetrahydropyridin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	230-232	413.3
9	94	7-(1-cyclobutyl-1,2,3,6-tetrahydropyridin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	252-254	427.3
9	95	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-propyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	226-228	415.3
13*	96	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(piperidin-4-yl)-1H-isochromen-1-one	218-220	375.2
9	97	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one	294-296	432.3
9	98	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(oxetan-3-yl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one	253-255	429.3
6	99	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	221-226	396.3
6	100	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	291-296	410.3
6	101	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	286-291	438.3
6	102	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one	229-234	424.3
4	103	3-(2-methyl-1,3-benzothiazol-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	271-276	378.3
4	104	3-(2-methyl-1,3-benzothiazol-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	231-236	392.3
4	105	3-(2-methyl-1,3-benzothiazol-6-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	222-228	420.3
4	106	3-(2-methyl-1,3-benzothiazol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	229-235	378.2
9	107	3-(2-methyl-1,3-benzothiazol-6-yl)-7-[4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one	222-228	434.4
6	108	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	251-258	410.2
6	109	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	251-258	410.2
6	110	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	261-266	424.2
9	111	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one	271-276	424.2
9	112	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one	270-275	424.2

TABLE 1-continued

Ex	Cpd	Name	M.P.	MS
12	113	3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	269-273	393.2
9	114	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-methylpiperidin-4-yl)-1H-isochromen-1-one	248-250	389.4
9	115	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-ethylpiperidin-4-yl)-1H-isochromen-1-one	215-217	403.4
9	116	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-propylpiperidin-4-yl)-1H-isochromen-1-one	204-206	417.5
9	117	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(propan-2-yl)piperidin-4-yl]-1H-isochromen-1-one	227-229	417.5
9	118	7-(1-cyclobutylpiperidin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	ND	429.5
9	119	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(oxetan-3-yl)piperidin-4-yl]-1H-isochromen-1-one	225-227	431.5
9	120	7-[(3S)-4-ethyl-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	255-260	404.2
9	121	7-[(3S)-3,4-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	250-255	390.2
6	122	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	279-285	376.2
6	123	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-hydroxypiperidin-1-yl)-1H-isochromen-1-one	290-292	391.3
6	124	7-[4-(dimethylamino)piperidin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	274-276	418.3
6	125	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	219-225	376.2
9	126	7-[(3R)-4-ethyl-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	238-241	404.2
9	127	7-[(3R)-3,4-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	243-248	390.3
6	128	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	231-236	404.3
9	129	7-[(3R,5S)-4-ethyl-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	233-238	418.2
9	130	7-[(3R,5S)-4-(2-hydroxyethyl)-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	241-245	434.2
9	131	3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one	279-284	404.3
9	132	7-[(3R,5S)-4-cyclobutyl-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	ND	444.3
9	133	7-[(3R)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	ND	420.2
9	134	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	ND	434.2
9	135	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-ethyl-3-methylpiperazin-1-yl]-1H-isochromen-1-one	238-243	418.2
9	136	7-[(3S)-4-cyclobutyl-3-methylpiperazin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	277-282	444.2
9	137	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one	249-254	432.3
9	138	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	266-271	432.2
9	139	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one	251-256	446.3
9	140	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-ethyl-3-methylpiperazin-1-yl]-1H-isochromen-1-one	243-246	418.2
9	141	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one	251-256	432.3
9	142	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	256-261	434.2
9	143	7-[(3R)-4-cyclobutyl-3-methylpiperazin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	281-285	444.3
9	144	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methyl-4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one	262-267	432.2
11	145	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-(2-methoxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	205-210	448.2
11	146	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-(2-methoxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	221-226	448.2
9	147	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-4-ethyl-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one	253-258	432.2
9	148	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one	259-263	446.3
9	149	3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one	261-266	418.2
6	150	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	ND	390.2
6	151	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	229-234	404.3

TABLE 1-continued

Ex	Cpd	Name	M.P.	MS
6	152	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	206-211	404.3
6	153	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one	208-213	404.3
6	154	7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	239-244	418.2
9	155	7-[(3S)-3,4-dimethylpiperazin-1-yl]-3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one	233-238	418.2
4	156	3-(2-methyl-2H-indazol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one	268-270	361.3
4	157	3-(2-methyl-2H-indazol-5-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one	239-240	375.3
9	158	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one	235-238	432.2
12	159	3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one	239-241	387.3

or a salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

BIOLOGICAL EXAMPLES

To describe in more detail and assist in understanding the present description, the following non-limiting biological examples are offered to more fully illustrate the scope of the description and are not to be construed as specifically limiting the scope thereof. Such variations of the present description that may be now known or later developed, which would be within the purview of one skilled in the art to ascertain, are considered to fall within the scope of the present description and as hereinafter claimed. These examples illustrate the testing of certain compounds described herein in vitro and/or in vivo and demonstrate the usefulness of the compounds for treating of SMA by enhancing the inclusion of exon 7 of SMN2 into mRNA transcribed from the SMN2 gene. Compounds of Formula (I) enhance inclusion of exon 7 of SMN2 into mRNA transcribed from the SMN2 gene and increase levels of Smn protein produced from the SMN2 gene, and thus can be used to treat SMA in a human subject in need thereof.

Example 1

SMN2 Minigene Construct

Preparation of the Minigene Constructs

DNA corresponding to a region of the SMN2 gene starting from the 5' end of exon 6 (ATAATTCCCC) (SEQ ID NO. 14) and ending at nucleic acid residue 23 of exon 8 (CAG-CAC) (SEQ ID NO. 15) was amplified by PCR using the following primers:

(SEQ ID NO. 16)
Forward primer: 5'-CGCGGATCCATAATTCCCCACACCTC-3'

(SEQ ID NO. 17)
Reverse primer: 5'-CGCGGATCCGTGCTGCTCTATGCCAGCA-3'

The 5' end of each primer was designed to add a BamHI restriction endonuclease recognition site at both the 5' end of exon 6 (GGATCC) (SEQ ID NO. 18) and the 3' end after the 23rd nucleotide of exon 8. Using the BamHI restriction endonuclease recognition sites, the PCR fragment was cloned into a derivative of the original pcDNA 3.1/Hygro vector which was modified as disclosed in United States Patent Publication US20050048549.

New UTRs were added to the modified vector using the HindIII site and the BamHI restriction sites comprising a 5'DEG UTR: 5'-TAGCTTCTTACCCGTACTCCACCGT-TGGCAGCACGATCGCACGTCCCACGTGAAC CAT-TGGTAAACCCTG-3' (SEQ ID NO. 19) cloned into the modified pcDNA3.1/Hygro vector together with a start codon upstream of the BamHI restriction site and;

a 3'DEG UTR: 5'-ATCGAAAGTACAGGACTAGCCT-TCCCTAGCAACCGCGGGCTGGGAGTCTGAGACAT CACTCAAGATATATGCTCGGTAACGTAT-GCTCTAGCCATCTAACTATTCCCTATGTCT TAT-AGGG-3' (SEQ ID NO. 20) cloned into the modified pcDNA3.1/Hygro vector using the NotI restriction endonuclease recognition site and the XhoI restriction endonuclease recognition site with a stop codon immediately downstream of the NotI restriction site. In addition, a firefly luciferase gene lacking the start codon was cloned into the vector using the BamHI and NotI restriction sites.

The resulting minigene comprises, in 5' to 3' order: the 5'-DEG UTR, the start codon, six additional nucleotides forming a BamHI restriction site, the nucleic acid residues of exon 6, the nucleic acid residues of intron 6 of SMN2, the nucleic acid residues of exon 7 of SMN2, the nucleic acid residues of intron 7 of SMN2, and the first 23 nucleic acid residues of exon 8 of SMN2, an additional six nucleotides forming a BamHI restriction site and the firefly luciferase gene lacking the start codon.

A single adenine residue was inserted after nucleotide 48 of exon 7 of SMN2 by site-directed mutagenesis. This minigene construct is referred to as SMN2-A.

SMN2 transcripts derived from minigenes containing exon 6 through 8 and the intervening introns recapitulate the splicing of their endogenous pre-mRNA (Lorson et al, Proc. Natl. Acad. Sci. U.S.A., 1999, 96 (11), 6307). An SMN2-alternative splicing reporter construct which contains exons 6 through 8 and the intervening introns followed by a luciferase reporter gene was generated. Salient features of this construct are the lack of the start codon in the luciferase gene, inactivation of the termination codon (in the open reading frame that encodes the SMN protein) of exon 7 by insertion of a nucleotide after nucleic acid 48 of exon 7 and addition of a start codon (ATG) immediately upstream of exon 6. A single adenine (SMN2-A) was inserted after nucleic residue 48 of exon 7.

The SMN2 minigene was designed such that the luciferase reporter is in frame with the ATG start codon immediately upstream of exon 6 when exon 7 is present in the mRNA and the luciferase reporter is out of frame with the ATG start

codon immediately upstream of exon 6 if exon 7 of SMN2 is removed during splicing of the pre-mRNA. In addition, in the absence of exon 7, the open reading frame that starts from the ATG start codon immediately upstream of exon 6 contains a stop codon in the fragment of exon 8 of SMN. Thus, in the presence of compounds that increase the inclusion of exon 7 of SMN2 into mRNA transcribed from the SMN2 gene, more transcripts containing exon 7 and more functional reporter are produced. A schematic illustration of this description can be found in FIG. 1.

The DNA sequence of the minigene from the SMN2-A construct SEQ ID NO. 21 is provided in FIG. 2a. A picture of the minigene SMN2-A subsequences is shown in FIG. 2b.

Example 2

SMN2 Minigene mRNA Splicing RT-qPCR Assay in Cultured Cells

The reverse transcription-quantitative PCR-based (RT-qPCR) assay is used to quantify the level of the full length SMN2 minigene mRNA containing SMN2 exon 7 in a HEK293H cell line stably transfected with said minigene and treated with a test compound.

Materials

Material	Source
HEK293H cells	ATCC Catalog No.: CRL-1573
Cells-To-Ct lysis buffer	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4399002
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
96-well flat-bottom plates	Becton Dickinson Catalog No.: 353072
RT-PCR Enzyme Mix	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388520 (also included in AgPath-ID kit Catalog No.: 4387391)
RT-PCR buffer	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388519 (also included in AgPath-ID kit Catalog No.: 4387391)
AgPath-ID One-Step RT-PCR kit	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4387391
Thermocycler	Life Technologies, Inc. (formerly Applied Biosystems) 7900HT

Protocol.

HEK293H cells stably transfected with the SMN2-A minigene construct described above (10,000 cells/well) are seeded in 200 μ L of cell culture medium (DMEM plus 10% FBS, with 200 μ g/mL hygromycin) in 96-well flat-bottom plates and the plate is immediately swirled to ensure proper dispersal of cells, forming an even monolayer of cells. Cells are allowed to attach for at least 4-6 hours. Test compounds are serially diluted 3.16-fold in 100% DMSO to generate a 7-point concentration curve. A solution of test compound (1 μ L, 200 \times in DMSO) is added to each cell-containing well and the plate is incubated for 24 hours in a cell culture incubator (37° C., 5% CO₂, 100% relative humidity). 2 replicates are prepared for each test compound concentration. The cells are then lysed in Cells-To-Ct lysis buffer and the lysate is stored at -80° C.

Full length SMN2-A minigene and GAPDH mRNA are quantified using the following primers and probes provided in Table 3. Primer SMN Forward A (SEQ ID NO. 1) hybridizes to a nucleotide sequence in exon 7 (nucleotide 22 to nucleotide 40), primer SMN Reverse A (SEQ ID NO. 2) hybridizes to a nucleotide sequence in the coding sequence of Firefly luciferase, SMN Probe A (SEQ ID NO. 3) hybridizes to a

nucleotide sequence in exon 7 (nucleotide 50 to nucleotide 54) and exon 8 (nucleotide 1 to nucleotide 21). The combination of these three oligonucleotides detects only SMN1 or SMN2 minigenes (RT-qPCR) and will not detect endogenous SMN1 or SMN2 genes.

TABLE 3

Primers/ Probes	Sequence	Source
SMN Forward Primer A	SEQ ID NO. 1: GAAGGAAGGT-GCTCA CATT	PTC ¹
SMN Reverse Primer A	SEQ ID NO. 2: TCTTTAT-GTTTTGG CGTCTTC	PTC ¹
SMN Forward Probe A	SEQ ID NO. 3: 6FAM-AAGGAGA AATGCTGGCATAGAGCAGC-TAMRA	PTC ¹
hGAPDH Forward Probe	SEQ ID NO. 4: VIC-CGCCTGGTCA CCAGGGCTGCT-TAMRA	LTI ²
hGAPDH Forward Primer	SEQ ID NO. 5: CAACGGATTGGTC GTATTGG	LTI ²
hGAPDH Reverse Primer	SEQ ID NO. 6: TGATGGCAACAATA TCCACTTTACC	LTI ²

¹Primers and probes designed by PTC Therapeutics, Inc.;

²Commercially available from Life Technologies, Inc. (formerly Invitrogen).

The SMN forward and reverse primers are used at final concentrations of 0.4 μ M. The SMN probe is used at a final concentration of 0.15 μ M. The GAPDH primers are used at final concentrations of 0.2 μ M and the probe at 0.15 μ M.

The SMN2-minigene GAPDH mix (15 μ L total volume) is prepared by combining 7.5 μ L of 2 \times RT-PCR buffer, 0.4 μ L of 25 \times RT-PCR enzyme mix, 0.75 μ L of 20 \times GAPDH primer-probe mix, 4.0075 μ L of water, 2 μ L of 10-fold diluted cell lysate, 0.06 μ L of 100 μ M SMN forward primer, 0.06 μ L of 100 μ M SMN reverse primer, and 0.225 μ L of 100 μ M SMN probe.

PCR is carried out at the following temperatures for the indicated time: Step 1: 48° C. (15 min); Step 2: 95° C. (10 min); Step 3: 95° C. (15 sec); Step 4: 60° C. (1 min); then repeat Steps 3 and 4 for a total of 40 cycles.

Each reaction mixture contains both SMN2-A minigene and GAPDH primers/probe sets (multiplex design), allowing simultaneous measurement of the levels of two transcripts.

Two SMN spliced products are generated from the SMN2 minigene. The first spliced product containing exon 7, corresponding to full length SMN2 mRNA, is referred to herein using the term "SMN2 minigene FL." The second spliced product lacking exon 7 is referred to herein using the term "SMN2 minigene Δ 7."

The increase of SMN2 minigene FL mRNA relative to that in cells treated with vehicle control is determined from real-time PCR data using a modified $\Delta\Delta$ Ct method (as described in Livak and Schmittgen, Methods, 2001, 25:402-8). The amplification efficiency (E) is calculated from the slope of the amplification curve for SMN2 minigene FL and GAPDH individually. The abundances of SMN2 minigene FL and GAPDH are then calculated as $(1+E)^{-Ct}$, where Ct is the threshold value for each amplicon. The abundance of SMN2 minigene FL is normalized to GAPDH abundance. The normalized SMN2 minigene FL abundance from test compound-treated samples is then divided by normalized SMN2 mini-

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gene FL abundance from vehicle-treated cells to determine the level of SMN2 FL mRNA relative to vehicle control.

Results.

As seen in FIG. 3, cells treated with Compound 65 (FIG. 3a) and Compound 69 (FIG. 3b) increased SMN2 minigene FL mRNA at low concentrations. The two test compounds fully restored exon 7 inclusion relative to untreated cells.

For compounds of Formula (I) or a form thereof disclosed herein, Table 4 provides the EC_{1.5x} for production of full length SMN2 mRNA that was obtained from the 7-point concentration data generated for each test compound according to the procedure of Biological Example 2. The term “EC_{1.5x} for production of full length SMN2 mRNA” is defined as that concentration of test compound that is effective in increasing the amount of full length SMN2 mRNA to a level 1.5-fold greater relative to that in vehicle-treated cells. An EC_{1.5x} for production of full length SMN2 mRNA between >3 μM and ≤30 μM is indicated by one star (*), an EC_{1.5x} between >1 μM and ≤3 μM is indicated by two stars (**), an EC_{1.5x} between >0.3 μM and ≤1 μM is indicated by three stars (***), an EC_{1.5x} between >0.1 μM and ≤0.3 μM is indicated by four stars (****) and an EC_{1.5x} ≤0.1 μM is indicated by five stars (*****).

TABLE 4

Cpd	EC _{1.5x}
1	*
2	***
3	*****
4	*
5	*
6	*
7	*
8	*
9	*
10	*
11	*
12	*
13	*
14	*
15	*
16	***
17	***
18	***
19	*
20	*
21	*
22	*
23	*
24	***
25	*
26	*
27	*
28	***
29	***
30	*****
31	*****
32	***
33	*
34	***
35	***
36	*
37	*
38	*
39	*
40	***
41	***
42	***
43	***
44	*
45	***
46	***
47	*
48	***

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TABLE 4-continued

Cpd	EC _{1.5x}
49	*
50	*****
51	*
52	***
53	***
54	***
55	***
56	***
57	***
58	***
59	*
60	***
61	*
62	***
63	***
64	***
65	***
66	***
67	*
68	***
69	***
70	***
71	***
72	***
73	***
74	***
75	***
76	***
77	***
78	***
79	***
80	***
81	***
82	***
83	***
84	***
85	***
86	***
87	***
88	***
89	***
90	***
91	***
92	***
93	***
94	***
95	***
96	***
97	*
98	***
99	***
100	***
101	***
102	***
103	***
104	***
105	***
106	*
107	***
108	***
109	***
110	***
111	***
112	*
113	***
114	***
115	***
116	***
117	***
118	***
119	***
120	***
121	***
122	***
123	*
124	***
125	***
126	***

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TABLE 4-continued

Cpd	EC _{1.5x}
127	*****
128	*****
129	*****
130	*****
131	*****
132	*****
133	*****
134	*****
135	*****
136	*****
137	*****
138	*****
139	*****
140	*****
141	*****
142	*****
143	*****
144	*****
145	*****
146	*****
147	*****
148	*****
149	*****
150	*****
151	*****
152	*****
153	*****
154	*****
155	*****
156	*****
157	*****
158	*****
159	*****

Example 3

Endogenous SMN2 mRNA RT-qPCR Splicing Assay
in Cultured Cells

The reverse transcription-quantitative PCR-based (RT-qPCR) assay is used to quantify the levels of the full length and $\Delta 7$ SMN2 mRNA in primary cells and cell lines containing the SMN2 gene treated with a test compound.

Materials

Material	Source
SMA Type 1 human cells	GM03813 (Coriell Institute)
Cells-To-Ct lysis buffer	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4399002
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
96-well flat-bottom plates	Becton Dickinson Catalog No.: 353072
RT-PCR Enzyme Mix	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388520 (also included in AgPath-ID kit Catalog No.: 4387391)
RT-PCR buffer	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388519 (also included in AgPath-ID kit Catalog No.: 4387391)
AgPath-ID One-Step RT-PCR kit	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4387391
Thermocycler	Life Technologies, Inc. (formerly Applied Biosystems) 7900HT

Protocol.

GM03813 SMA patient cells (5,000 cells/well) are seeded in 200 μ L of cell culture medium (DMEM plus 10% FBS) in 96-well flat-bottom plates and the plate is immediately swirled to ensure proper dispersal of cells, forming an even

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monolayer of cells. Cells are allowed to attach for at least 4-6 hrs. Test compounds are serially diluted 3.16-fold in 100% DMSO to generate a 7-point concentration curve. A solution of test compound (1 μ L, 200 \times in DMSO) is added to each test well and 1 μ L DMSO is added to each control well. The plate is incubated for 24 hrs in a cell culture incubator (37° C., 5% CO₂, 100% relative humidity). The cells are then lysed in Cells-To-Ct lysis buffer and the lysate is stored at 80° C.

SMN2-specific spliced products and GAPDH mRNA are identified using the following primers and probes in Table 5. Primer SMN FL Forward B (SEQ ID NO. 7) hybridizes to a nucleotide sequence in exon 7 (nucleotide 32 to nucleotide 54) and exon 8 (nucleotide 1 to nucleotide 4), primer SMN $\Delta 7$ Forward B (SEQ ID NO. 8) hybridizes to a nucleotide sequence in exon 6 (nucleotide 87 to nucleotide 111) and exon 8 (nucleotide 1 to nucleotide 3), primer SMN Reverse B (SEQ ID NO. 9) hybridizes to a nucleotide sequence in exon 8 (nucleotide 39 to nucleotide 62), probe SMN Probe B (SEQ ID NO. 10) hybridizes to a nucleotide sequence in exon 8 (nucleotide 7 to nucleotide 36). These primers and probe hybridize to nucleotide sequences common to human SMN1 and SMN2 mRNA. Since the SMA patient cells used in Example 3 contain only the SMN2 gene, RT-qPCR can quantify only SMN2 full-length and $\Delta 7$ mRNA.

TABLE 5

Primer/Probe	Sequence	Source
SMN FL Forward Primer B	SEQ ID NO. 7: GCTCACATTCCTTA AATTAAGGAGAAA	PTC ¹
SMN $\Delta 7$ Forward Primer B	SEQ ID NO. 8: TGGCTATCATACTG GCTATTATATGGAA	PTC ¹
SMN Reverse Primer B	SEQ ID NO. 9: TCCAGATCTGTCTG ATCGTTTCTT	PTC ¹
SMN Forward Probe B	SEQ ID NO. 10: 6FAM-CTGGCATA GAGCAGCACTAAATGACACCAC-TAMRA	PTC ¹
hGAPDH Forward Probe	SEQ ID NO. 4: VIC-CGCCTGGTCA CCAGGGCTGCT-TAMRA	LTI ²
hGAPDH Forward Primer	SEQ ID NO. 5: CAACGGATTGGTC GTATTGG	LTI ²
hGAPDH Reverse Primer	SEQ ID NO. 6: TGATGGCAACAATA TCCACTTTACC	LTI ²

¹Primers and probes designed by PTC Therapeutics, Inc.;

²Commercially available from Life Technologies, Inc. (formerly Invitrogen).

The SMN forward and reverse primers are used at final concentrations of 0.4 μ M. The SMN probe is used at a final concentration of 0.15 μ M. GAPDH primers are used at final concentrations of 0.1 μ M and the probe at 0.075 μ M. The One-Step RT-PCR kit was used as the Real-Time PCR Mix.

The SMN-GAPDH mix (10 μ L total volume) is prepared by combining 5 μ L of 2 \times RT-PCR buffer, 0.4 μ L of 25 \times RT-PCR enzyme mix, 0.25 μ L of 20 \times GAPDH primer-probe mix, 1.755 μ L water, 2.5 μ L of cell lysate, 0.04 μ L of 100 μ M SMN FL or SMN $\Delta 7$ forward primer, 0.04 μ L of 100 μ M SMN reverse primer, and 0.015 μ L of 100 μ M probe.

PCR is carried out at the following temperatures for the indicated time: Step 1: 48° C. (15 min); Step 2: 95° C. (10 min); Step 3: 95° C. (15 sec); Step 4: 60° C. (1 min); then, repeat Steps 3 and 4 for a total of 40 cycles.

Each reaction mixture contains either SMN2 FL and GAPDH or SMN2 $\Delta 7$ and GAPDH primers/probe sets (multiplex design), allowing simultaneous measurement of the levels of two transcripts.

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The endogenous SMN2 gene gives rise to two alternatively spliced mRNA. The full length SMN2 mRNA that contains exon 7 and is referred to herein using the term "SMN2 FL." The truncated mRNA that lacks exon 7 and is referred to herein using the term "SMN2 Δ7."

The increase of SMN2 FL and decrease in SMN2 Δ7 mRNA relative to those in cells treated with vehicle control are determined from real-time PCR data using a modified ΔΔCt method (as described in Livak and Schmittgen, Methods, 2001, 25:402-8). The amplification efficiency (E) is calculated from the slope of the amplification curve for SMN2 FL, SMN2 Δ7, and GAPDH individually. The abundances of SMN2 FL, SMN2 Δ7, and GAPDH are then calculated as $(1+E)^{-Ct}$, where Ct is the threshold value for each amplicon. The abundances of SMN2 FL and SMN2 Δ7 are normalized to GAPDH abundance. The normalized SMN2 FL and SMN2 Δ7 abundances from test compound-treated samples are then divided by normalized SMN2 FL and SMN2 Δ7 abundances, respectively, from vehicle-treated cells to determine the levels of SMN2 FL and SMN2 Δ7 mRNA relative to vehicle control.

Results.

As seen in FIG. 4, cells treated with increasing concentrations of Compound 65 (FIG. 4a) and Compound 69 (FIG. 4b) contain progressively more SMN2 FL mRNA and less SMN2 Δ7 mRNA than those treated with vehicle, indicating a correction of SMN2 alternative splicing.

Example 4

Endogenous SMN2 mRNA End-Point Semi-Quantitative RT-PCR Splicing Assay in Cultured Cells

The endpoint reverse transcription-PCR splicing assay is used to visualize and quantify the levels of the full length and Δ7 SMN2 mRNA in primary cells and cell lines containing the SMN2 gene treated with a test compound.

Materials

Material	Source
SMA Type 1 human cells	GM03813 (Coriell Institute)
Cells-To-Ct lysis buffer	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4399002
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
96-well flat-bottom plates	Becton Dickinson Catalog No.: 353072
Platinum Taq HiFi DNA Polymerase Super Mix	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11304-016
iScript RT enzyme kit	BioRad: Catalog No.: 170-8890
Ethidium bromide 2% agarose E gels 48-Well Double Comb	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: G8008-02
Gel Documentation System	UVP Gel Doc It 310 Imaging system

Protocol.

GM03813 SMA patient cells (5,000 cells/well) are seeded in 200 μL of cell culture medium (DMEM plus 10% FBS) in 96-well flat-bottom plates and the plate is immediately swirled to ensure proper dispersal of cells, forming an even monolayer of cells. Cells are allowed to attach for at least 4-6 hrs. Test compounds are serially diluted 3.16-fold in 100% DMSO to generate a 7-point concentration curve. A solution of test compound (1 μL, 200× in DMSO) is added to each test well and 1 μL DMSO is added to each control well. The plate is incubated for 24 hrs in a cell culture incubator (37° C., 5%

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CO₂, 100% relative humidity). The cells are then lysed in Cells-To-Ct lysis buffer and the lysate is stored at 80° C.

SMN FL and Δ7 mRNA are identified using the following primers in Table 6. These primers hybridize to a nucleotide sequence in exon 6 (SMN Forward C, SEQ ID NO. 11) (nucleotide 43 to nucleotide 63) and exon 8 (SMN Reverse C, SEQ ID NO. 12) (nucleotide 51 to nucleotide 73) common to human SMN1 and SMN2 mRNA. Since the SMA patient cells used in Example 4 contain only the SMN2 gene, RT-PCR can visualize and quantify only SMN2 full-length and SMN2 Δ7 mRNA.

TABLE 6

Primer	Sequence	Source
SMN Forward C	SEQ ID NO. 11: GATGCTGATGCTTTGGGAAGT	PTC ¹
SMN Reverse C	SEQ ID NO. 12: CGCTTCACATTCAGATCTGTC	PTC ¹

¹Primers designed by PTC Therapeutics, Inc.

To synthesize cDNA, 5 μL of lysate, 4 μL of 5× iScript reaction mix, 1 μL of reverse transcriptase, and 10 μL of water are combined and incubated 5 min at 25° C. followed by 30 min at 42° C., followed by 5 min at 85° C. The cDNA solution is stored at -20° C.

To perform endpoint PCR, 5 μL of cDNA, 0.2 μL of 100 μM forward primer, 0.2 μL of 100 μM reverse primer, and 22.5 μL of polymerase super mix are combined in a 96 well semiskirted PCR plate. PCR is carried out at the following temperatures for the indicated time: Step 1: 94° C. (2 min), Step 2: 94° C. (30 sec), Step 3: 55° C. (30 sec), Step 4: 68° C. (1 min), then repeat Steps 2 to 4 for a total of 33 cycles, then hold at 4° C.

10 μL of each PCR sample is electrophoretically separated on a 2% agarose E-gel for 14 minutes stained with double-stranded DNA (dsDNA) staining reagents (e.g., ethidium bromide) and visualized using a gel imager.

Results.

As seen in FIG. 5, cells treated with increasing concentrations of Compound 65 (FIG. 5a) and Compound 69 (FIG. 5b) contain progressively more SMN2 FL mRNA and less SMN2 Δ7 mRNA, indicating a correction of SMN2 alternative splicing.

Example 5

SMN2 mRNA RT-qPCR Splicing Assay in Animal Tissues

The reverse transcription-quantitative PCR-based (RT-qPCR) assay is used to quantify the levels of the full length and Δ7 SMN2 mRNA in tissues from mice treated with test compound.

Materials

Material	Source
Tissues from C/C-allele SMA mice	The Jackson Laboratory, strain No.: 008714 (B6.129-Smn ^{1tm5(Smn1/SMN2)Mrph/J})
Tissues from Δ7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1Msd/J})
RT-PCR Enzyme Mix	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388520 (also included in AgPath-ID kit Catalog No.: 4387391)

-continued

Material	Source
RT-PCR buffer	Life Technologies, Inc. (formerly Applied Biosystems) Part No.: 4388519 (also included in AgPath-ID kit Catalog No.: 4387391)
AgPath-ID One-Step RT-PCR kit	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4387391
Mouse GAPDH primers and probes	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4352339E
QIAzol Lysis Reagent	Qiagen Catalog No.: 79306
RNeasy Lipid Tissue Mini Kit	Qiagen Catalog No.: 74804
5 mm Stainless Steel Bead	Qiagen Catalog No.: 69989
TissueLyser II	Qiagen Catalog No.: 85300
Thermocycler	Life Technologies, Inc. (formerly Applied Biosystems) 7900HT

Protocol.

C/C-allele SMA mice are treated by oral gavage two times per day (BID) for 10 days with test compounds re-suspended in 0.5% HPMC and 0.1% Tween-80. Tissue samples were collected and snap frozen for RNA purification.

Tissue samples (20-40 mg) are homogenized in QIAzol Lysis Reagent for 2 minutes at 20 Hz in the TissueLyser II using one stainless steel bead. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitioned to the upper, aqueous phase is extracted and ethanol is added to provide appropriate binding conditions. The sample is then applied to the RNeasy spin column from the RNeasy Mini Kit, where total RNA binds to the membrane. The RNA is eluted in RNase-free water then stored at -20° C. and subsequently analyzed using the TaqMan RT-qPCR on the 7900HT Thermocycler. Total RNA is diluted ten fold and 2.5 μ L, of the diluted sample is added to the TaqMan RT-qPCR mixture.

SMN2 spliced products are identified using the following primers and probes in Table 7. Primer SMN FL Forward B (SEQ ID NO. 7) hybridizes to a nucleotide sequence in exons 7 and 8, primer SMN Δ 7 Forward B (SEQ ID NO. 8) hybridizes to a nucleotide sequence in exons 6 and 8, primer SMN Reverse B (SEQ ID NO. 9) hybridizes to a nucleotide sequence in exon 8, probe SMN Probe B (SEQ ID NO. 10) hybridizes to a nucleotide sequence in exon 8. These primers and probe hybridize to nucleotide sequences common to human SMN1 and SMN2 mRNA. Since the SMA patient cells used in Example 5 contain only the SMN2 gene, RT-qPCR can quantify only SMN2 full-length and Δ 7 mRNA.

TABLE 7

Primer/Probe	Sequence	Source
SMN FL Forward Primer B	SEQ ID NO. 7: GCTCACATTCTTAAATTAAGGAGAAA	PTC ¹
SMN Δ 7 Forward Primer B	SEQ ID NO. 8: TGGCTATCATACTGGCTATTATATGGAA	PTC ¹
SMN Reverse Primer B	SEQ ID NO. 9: TCCAGATCTGTCTGATCGTTTCTT	PTC ¹
SMN Forward Probe B	SEQ ID NO. 10: 6FAM-CTGGCATA GAGCAGCACTAAATGACACCAC-TAMRA	PTC ¹

¹Primers and probes designed by PTC Therapeutics, Inc.

The SMN forward and reverse primers are used at final concentrations of 0.4 μ M. The SMN probe is used at a final concentration of 0.15 μ M. The SMN-GAPDH Mix (10 μ L, total volume) is prepared by combining 5 μ L of 2 \times RT-PCR

buffer, 0.4 μ L of 25 \times RT-PCR enzyme mix, 0.5 μ L of 20 \times GAPDH primer-probe mix, 1.505 μ L of water, 2.5 μ L of RNA solution, 0.04 μ L of 100 μ M forward primer, 0.04 μ L of 100 μ M reverse primer, and 0.015 μ L of 100 μ M SMN probe.

Each PCR cycle was carried out at the following temperatures for the indicated time: Step 1: 48° C. (15 min); Step 2: 95° C. (10 min); Step 3: 95° C. (15 sec); Step 4: 60° C. (1 min); then, repeat Steps 3 and 4 for a total of 40 cycles.

Each reaction mixture contains either SMN2 FL and mGAPDH or SMN2 Δ 7 and mGAPDH primers/probe sets (multiplex design), allowing simultaneous measurement of the levels of two transcripts.

The increase of SMN2 FL and decrease in SMN2 Δ 7 mRNA relative to those in tissues from animals treated with vehicle control are determined from real-time PCR data using a modified $\Delta\Delta$ Ct method (as described in Livak and Schmittgen, Methods, 2001, 25:402-8). The amplification efficiency (E) is calculated from the slope of the amplification curve for SMN2 FL, SMN2 Δ 7, and GAPDH individually. The abundances of SMN2 FL, SMN2 Δ 7, and GAPDH are then calculated as $(1+E)^{-Ct}$, where Ct is the threshold value for each amplicon. The abundances of SMN2 FL and SMN2 Δ 7 are normalized to GAPDH abundance. The normalized SMN2 FL and SMN2 Δ 7 abundances from test compound-treated samples are then divided by normalized SMN2 FL and SMN2 Δ 7 abundances, respectively, from vehicle-treated cells to determine the levels of SMN2 FL and SMN2 Δ 7 mRNA relative to vehicle control.

Results.

As seen in FIG. 6, tissues of animals treated with Compound 65 (FIG. 6a) and Compound 69 (FIG. 6b) contain substantially more SMN2 FL mRNA and less SMN2 Δ 7 mRNA than those treated with vehicle, indicating a correction of SMN2 alternative splicing.

Example 6

Endogenous SMN2 mRNA End-Point
Semi-Quantitative RT-PCR Splicing Assay in Animal
Tissues

The endpoint reverse transcription-PCR (RT-PCR) splicing assay is used to quantify the levels of the full length and Δ 7 SMN2 mRNA in tissues from mice treated with test compound.

Materials

Material	Source
Tissues from C/C-allele SMA mice	The Jackson Laboratory, strain No.: 008714 (B6.129-Smn1 ^{emS(Smn1/SMN2)Mrph/J})
Tissues from Δ Exon7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1Msd/J})
Qiagen RNeasy lipid kit	Qiagen Catalog No.: 74804
Platinum Taq HiFi DNA Polymerase Super Mix	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11304-016
iScript RT enzyme kit	BioRad Catalog No.: 170-8890
Twin.tec 96-Well Semiskirted PCR Plate	Eppendorf Catalog No.: 951020389
Ethidium bromide 2% agarose E gels 48-Well Double Comb	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: G8008-02
Gel Documentation System	UVP Gel Doc It 310 Imaging system

Protocol.

C/C-allele SMA mice are treated by oral gavage BID for 10 days with test compounds in 0.5% HPMC and 0.1% Tween-80. Tissue samples are collected and snap frozen for RNA purification.

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Tissue samples (20-40 mg) are homogenized in QIAzol Lysis Reagent for 2 minutes at 20 Hz in the TissueLyser II using one stainless steel bead. After addition of chloroform, the homogenate is separated into aqueous and organic phases by centrifugation. RNA partitioned to the upper, aqueous phase is extracted and ethanol is added to provide appropriate binding conditions. The sample is then applied to the RNeasy spin column from the RNeasy Mini Kit, where total RNA binds to the membrane. The RNA is eluted in RNase-free water then stored at -20°C .

SMN2 spliced products are identified using the following amplification primers in Table 8. These primers hybridize to a nucleotide sequence in exon 6 (SMN Forward D, SEQ ID NO. 13) (nucleotide 22 to nucleotide 46) and exon 8 (SMN Reverse C, SEQ ID NO. 12), common to human SMN1 and SMN2 mRNA.

TABLE 8

Primer	Sequence	Source
SMN Forward D	SEQ ID NO. 13: ATATGTCCAGATTCTTGTGATG	PTC ¹
SMN Reverse C	SEQ ID NO. 12: CGCTTCACATTCCAGATCTGTC	PTC ¹

¹Primers designed by PTC Therapeutics, Inc.

To synthesize cDNA, combine 1 μL of RNA solution (25-50 ng), 4 μL of 5 \times iScript reaction mix, 1 μL of reverse transcriptase, and 10 μL of water are combined and incubates 25°C . for 5 min followed by 42°C . for 30 min followed by 85°C . for 5 min. The cDNA solution is stored at -20°C .

To perform endpoint PCR, 5 μL of cDNA, 0.2 μL of 100 μM forward primer, 0.2 μL of 100 μM reverse primer, and 22.5 μL of polymerase super mix are combined in a 96 well semiskirted PCR plate. PCR is carried out at the following temperatures for the indicated time: Step 1: 94°C . (2 min), Step 2: 94°C . (30 sec), Step 3: 55°C . (30 sec), Step 4: 68°C . (1 min), then repeat Steps 2 to 4 for a total of 33 cycles, then hold at 4°C .

10 μL of each PCR sample is electrophoretically separated on a 2% agarose E-gel for 14 minutes stained with dsDNA staining reagents (e.g., ethidium bromide) and visualized using a gel imager.

Results.

As seen in FIG. 7, tissues from mice treated with increasing concentrations of Compound 65 contain progressively more SMN2 FL mRNA and less SMN2 $\Delta 7$ mRNA, indicating a correction of SMN2 alternative splicing.

Example 7

Smn Protein Assay in Cultured Cells

The SMN HTRF (homogeneous time resolved fluorescence) assay is used to quantify the level of Smn protein in SMA patient fibroblast cells treated with test compounds. The results of the assay are shown in Table 9.

Materials

Material	Source
SMA Type 1 human cells	GM03813 (Coriell Institute)
Protease inhibitor cocktail	Roche Applied Science Catalog No.: 11836145001
Anti-SMN d2	Blue cap Cisbio Catalog No.: 63IDC002-SMN

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-continued

Material	Source
Anti-SMN kryptate	Red cap Cisbio Catalog No.: 63IDC002-SMN
SMN reconstitution buffer	Cisbio Catalog No.: 63IDC002-SMN-Buffer
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
RIPA Lysis Buffer	20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% Sodium deoxycholate
Diluent Buffer	20 mM Tris-HCl pH 7.5, 150 mM NaCl
Envision Plate Reader	Perkin Elmer Model No.: 2103

Protocol.

Cells are thawed and cultured in DMEM-10% FBS for 72 hours. Cells are trypsinized, counted and re-suspended to a concentration of 25,000 cells/mL in DMEM-10% FBS. The cell suspensions are plated at 5,000 cells per well in a 96 well microtiter plate and incubated for 3 to 5 hours. To provide a control signal, three (3) wells in the 96 well plate do not receive cells and, thus, serve as Blank control wells. Test compounds are serially diluted 3.16-fold in 100% DMSO to generate a 7-point concentration curve. 1 μL of test compound solution is transferred to cell-containing wells and cells are incubated for 48 hours in a cell culture incubator (37°C ., 5% CO_2 , 100% relative humidity). Triplicate samples are set up for each test compound concentration. After 48 hours, the supernatant is removed from the wells and 25 μL of the RIPA lysis buffer, containing protease inhibitors, is added to the wells and incubated with shaking at room temperature for 1 hour. 25 μL of the diluent is added and then 35 μL of the resulting lysate is transferred to a 384-well plate, where each well contains 5 μL of the antibody solution (1:100 dilution of anti-SMN d2 and anti-SMN kryptate in SMN reconstitution buffer). The plate is centrifuged for 1 minute to bring the solution to the bottom of the wells, then incubated overnight at room temperature. Fluorescence for each well of the plate at 665 nm and 620 nm is measured on an EnVision multilabel plate reader (Perkin-Elmer).

The normalized fluorescence signal is calculated for each sample, Blank and vehicle control well by dividing the signal at 665 nm by the signal at 620 nm. Normalizing the signal accounts for possible fluorescence quenching due to the matrix effect of the lysate. The ΔF value (a measurement of Smn protein abundance as a percent value) for each sample well is calculated by subtracting the normalized average fluorescence for the Blank control wells from the normalized fluorescence for each sample well, then dividing this difference by the normalized average fluorescence for the Blank control wells and multiplying the resulting value by 100. The ΔF value for each sample well represents the Smn protein abundance from test compound-treated samples. The ΔF value for each sample well is divided by the ΔF value for the vehicle control wells to calculate the fold increase in Smn protein abundance relative to the vehicle control.

Results.

As seen in FIG. 8, SMA Type 1 patient fibroblast cells treated with Compound 65 (FIG. 8a) and Compound 69 (FIG. 8b) show a dose dependent increase in Smn protein expression as measured by the SMN HTRF assay.

For compounds of Formula (I) or a form thereof disclosed herein, Table 9 provides the $\text{EC}_{1.5\%}$ for Smn protein expression that was obtained from the 7-point concentration data generated for each test compound according to the procedure of Biological Example 7. The term " $\text{EC}_{1.5\%}$ for Smn protein expression" is defined as that concentration of test compound that is effective in producing 1.5 times the amount of Smn

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protein in an SMA patient fibroblast cell compared to the amount produced from the DMSO vehicle control. An $EC_{1.5\times}$ for Smn protein expression between $>3\text{ }\mu\text{M}$ and $<10\text{ }\mu\text{M}$ is indicated by one star (*), an $EC_{1.5\times}$ between $>1\text{ }\mu\text{M}$, and $\leq 3\text{ nM}$ is indicated by two stars (**), an $EC_{1.5\times}$ between $>0.3\text{ nM}$ and $\leq 1\text{ nM}$ is indicated by three stars (***) and an $EC_{1.5\times}\leq 0.3\text{ nM}$ is indicated by four stars (****).

TABLE 9

Cpd	$EC_{1.5\times}$
3	****
24	****
30	****
31	***
34	*
43	****
46	****
50	***
52	*
56	****
57	****
58	****
62	****
63	****
64	****
65	****
66	****
67	***
68	*
69	****
70	****
71	****
72	****
73	****
74	****
75	****
76	****
77	****
79	****
80	****
81	****
82	****
83	****
84	****
85	****
87	****
88	****
89	****
90	****
91	****
92	****
93	****
94	****
95	****
96	****
98	****
99	****
100	****
101	****
103	****
108	****
109	****
110	****
111	****
112	****
113	****
114	****
115	****
116	****
117	****
118	****
119	****
120	****
121	****
122	****
124	****
125	****
126	****

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TABLE 9-continued

Cpd	$EC_{1.5\times}$
127	****
128	****
129	****
130	****
131	****
132	****
133	****
134	****
135	****
136	****
137	****
138	****
139	****
140	****
141	****
142	****
143	****
144	****
145	****
146	****
147	****
148	****
149	****
150	****
151	****
152	****
153	****
154	****
155	****
156	****
157	****
158	****
159	****

For compounds of Formula (I) or a form thereof disclosed herein, Table 10 provides the maximum fold (Fold) increase of Smn protein that was obtained from the 7-point concentration data generated for each test compound according to the procedure of Biological Example 7. A maximum fold increase of ≤ 1.2 is indicated by one star (*), a fold increase between >1.2 and ≤ 1.35 is indicated by two stars (**), a fold increase between >1.35 and ≤ 1.5 is indicated by three stars (***), a fold increase between >1.5 and ≤ 1.65 is indicated by four stars (****) and a fold increase >1.65 is indicated by five stars (*****).

TABLE 10

Cpd	Fold
1	***
2	***
3	*****
4	*
5	*
6	*
7	*
8	*
9	*
10	*
11	*
12	***
13	*
14	***
15	*
16	****
17	****
18	*
19	*
20	*
21	*
22	*
23	*
24	****

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TABLE 10-continued

Cpd	Fold
25	*
26	*
27	*
28	***
29	*
30	****
31	****
32	**
33	*
34	***
35	*
36	*
37	*
38	*
39	*
40	*
41	***
42	**
43	***
44	**
45	***
46	****
47	*
48	*
49	*
50	***
51	*
52	***
53	**
54	*
55	**
56	****
57	****
58	****
59	**
60	***
61	*
62	****
63	****
64	****
65	****
66	****
67	***
68	****
69	****
70	****
71	****
72	****
73	****
74	****
75	****
76	***
77	***
78	***
79	****
80	****
81	****
82	****
83	****
84	****
85	****
86	***
87	****
88	****
89	****
90	****
91	***
92	****
93	****
94	****
95	****
96	****
97	***
98	****
99	****
100	****
101	****
102	***

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TABLE 10-continued

Cpd	Fold
103	****
104	****
105	**
106	*
107	***
108	****
109	****
110	****
111	****
112	****
113	****
114	****
115	****
116	****
117	****
118	****
119	****
120	****
121	****
122	****
123	*
124	****
125	****
126	****
127	****
128	****
129	****
130	****
131	****
132	****
133	****
134	****
135	****
136	****
137	****
138	****
139	****
140	****
141	****
142	****
143	****
144	****
145	****
146	****
147	****
148	****
149	****
150	****
151	****
152	****
153	****
154	****
155	****
156	****
157	****
158	****
159	****

Example 8

Gems Count (Smn-Dependent Nuclear Speckle Count) Assay

The level of Smn protein directly correlates with the amount of nuclear foci, also known as gems, produced upon staining the cell with a fluorescently labeled anti-Smn antibody (Liu and Dreyfuss, EMBO J., 1996, 15:3555). Gems are multi-protein complexes whose formation is nucleated by the Smn protein and the gems count assay is used to evaluate the level of Smn protein in the cell. As described herein, the gems count assay is used to quantify the level of Smn protein in SMA patient fibroblast cells treated with a test compound.

Material	Source
SMA Type 1 human cells	GM03813 (Coriell Institute)
Primary Antibody- mouse anti-SMN clone 2B1	Sigma Catalog No.: S2944
Secondary Antibody- anti-mouse Alexa Fluor 555	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: A21422
Bovine Serum Albumin (BSA)	Sigma Catalog No.: A3294
4% Paraformaldehyde	Electron Microscopy Sciences Catalog No.: 15710
Bortezomib	LC Labs, Catalog No.: B-1408
0.05% Triton X-100	Sigma Catalog No.: 93443-100 mL
Mounting medium- ProLong	Life Technologies, Inc. (formerly Invitrogen) Catalog Nos.: P7481 and P36935
Gold Antifade Reagent with DAPI	Fisher Catalog No.: 12-548-B
22 × 22 No.: 1 sterile Cover slips	
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
PBS	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 10010-031
Clear-coat nail polish	Revlon brand Catalog No.: 1271-76
Zeiss Axovert 135	Zeiss
Fluorescence microscope	

Protocol:

Cells are thawed and incubated in DMEM-10% FBS for 72 hours, then trypsinized, counted and resuspended to 100,000 cells/mL in DMEM-10% FBS. 2 mL of the cell suspension is plated in a 6-well cell culture plate with a sterile cover slip and incubated for 3 to 5 hours. Test compounds are serially diluted 3.16-fold in 100% DMSO to generate a 7-point dilution curve. 10 μ L of test compound solution is added to each cell-containing well and incubated for 48 hours in a cell culture incubator (37° C., 5% CO₂, 100% relative humidity). Duplicates are set up for each test compound concentration. Cells containing DMSO at a final concentration of 0.5% are used as controls.

Cell culture medium is aspirated from the wells containing cover slips and gently washed three times with cold PBS. The cells are fixed by incubation for 20 minutes at room temperature while in paraformaldehyde. The cells are then washed two times with cold PBS followed by incubation for 5 minutes at room temperature with 0.05% Triton X-100 in PBS to permeabilize the cells. After the fixed cells are washed three times with cold PBS, they are blocked with 10% FBS for 1 hour. 60 μ L of primary antibody diluted 1:1000 in blocking buffer is added and the mixture is incubated for one hour at room temperature. The cells are washed three times with PBS and 60 μ L of secondary antibody diluted 1:5000 in blocking buffer is added, then the mixture is incubated for one hour at room temperature. The cover slips are mounted onto the slides with the aid of mounting medium and allowed to dry overnight. Nail polish is applied to the sides of the cover slip and the slides are stored, protected from light. A Zeiss Axovert 135 with a 63× Plan-Apochromat, NA=1.4 objective is used for immunofluorescence detection and counting. The number of gems is counted per \geq 150 nuclei and % activation is calculated using DMSO and 10 nM bortezomib as controls. For each test compound, the cells are examined at all wavelengths to identify test compounds with inherent fluorescence.

Results.

As seen in FIG. 9, SMA Type 1 patient cells treated with Compound 65 (FIG. 9a) and Compound 69 (FIG. 9b) contain progressively more gems relative to cells treated with DMSO.

Smn Protein Assay in Human Motor Neurons

Smn immunofluorescent confocal microscopy is used to quantify the level of Smn protein in human motor neurons treated with test compounds.

Protocol.

Human motor neurons derived from SMA iPS cells (Ebert et al., Nature, 2009, 457:2770; and, Rubin et al., BMC Biology, 2011, 9:42) are treated with test compound at various concentrations for 72 hours. The level of Smn protein in the cell nucleus is quantified using Smn immunostaining and confocal fluorescence microscopy essentially as described in Makhortova et al., Nature Chemical Biology, 2011, 7:544. The level of Smn protein in compound-treated samples is normalized to that in vehicle-treated samples and plotted as a function of the compound concentration.

Results.

As seen in FIG. 10, human motor neurons treated for 72 hours with increasing concentrations of Compound 65 (FIG. 10a) and Compound 69 (FIG. 10b) contain progressively more Smn protein in the nucleus.

Example 10

Smn Protein Assay in Animal Tissues

This Smn protein assay compares tissues from test compound treated mice with those from DMSO vehicle treated mice to determine the increase in levels of Smn protein produced from the human SMN2 gene.

Materials

Material	Source
Tissues from C/C-allele SMA mice	The Jackson Laboratory, strain No.: 008714 (B6.129-Smn1 ^{tm5(Smn1/SMN2)Mrph/J})
Tissues from A7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1Msd/J})
Protease inhibitor cocktail	Roche Applied Science Catalog No.: 11836145001
Anti-SMN d2	Blue cap Cisbio Catalog No.: 63IDC002-SMN
Anti-SMN kryptate	Red cap Cisbio Catalog No.: 63IDC002-SMN
SMN reconstitution buffer	Cisbio Catalog No.: 63IDC002-SMN-Buffer
RIPA Lysis Buffer	20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1% Sodium deoxycholate
Diluent Buffer	20 mM Tris-HCl pH 7.5, 150 mM NaCl
BCA protein assay kit	Pierce Catalog No.: 23225
White 384 well plate	Nunc Catalog No.: 351190
Polypropylene V-bottom plate	Falcon Catalog No.: 165195
Clear 96 well polystyrene plate	Nunc Catalog No.: 442404
5 mm Stainless Steel	Qiagen Catalog No.: 69989
Beads	
Safe-Lock Tubes 2.0 mL	Eppendorf Catalog No.: 022363352
Twin.tec 96-Well Semiskirted PCR Plate	Eppendorf Catalog No.: 951020389
TissueLyzer II	Qiagen Catalog No.: 85300
Envision Plate Reader	Perkin Elmer Model No.: 2103

Protocol.

The tissue samples in Safe-Lock tubes are weighed and the volume of RIPA buffer containing the protease inhibitor cocktail is added based on the weight to volume ratios for each type of tissue: Brain (50 mg/mL), Muscle (50 mg/mL) and Spinal Cord (25 mg/mL).

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Tissues are homogenized using the TissueLyzer by bead milling. 5 mm stainless steel beads are added to the sample and shaken vigorously for 5 minutes at 30 Hz in the Tissue-Lyzer. The samples are then centrifuged for 20 minutes at 14,000×g in a microcentrifuge and the homogenates transferred to the PCR plate. The homogenates are diluted in RIPA buffer to approximately 1 mg/mL for HTRF and approximately 0.5 mg/mL for total protein measurement using the BCA protein assay. For the SMN HTRF assay, 35 µL of the tissue homogenate is transferred to a 384-well plate containing 5 µL of the antibody solution (1:100 dilution of each of the anti-SMN2 and anti-SMN Kryptate in reconstitution buffer). To provide a control signal, three (3) wells in the plate contain only RIPA Lysis Buffer and, thus, serve as Blank control wells. The plate is centrifuged for 1 minute to bring the solution to the bottom of the wells and then incubated overnight at room temperature. Fluorescence for each well of the plate at 665 nm and 620 nm is measured on an EnVision multilabel plate reader (Perkin-Elmer). The total protein in the tissue homogenate is measured using the BCA assay according to the manufacturer's protocol.

The normalized fluorescence signal is calculated for each sample, Blank and vehicle control well by dividing the signal at 665 nm by the signal at 620 nm. Normalizing the signal accounts for possible fluorescence quenching due to the matrix effect of the tissue homogenate. The ΔF value (a measurement of Smn protein abundance as a percent value) for each tissue sample well is calculated by subtracting the normalized average fluorescence for the Blank control wells from the normalized fluorescence for each tissue sample well, then dividing this difference by the normalized average fluorescence for the Blank control wells and multiplying the resulting value by 100. The ΔF value for each tissue sample well is divided by the total protein quantity (determined using the BCA assay) for that tissue sample. The change in Smn protein abundance for each tissue sample relative to the vehicle control is calculated as the percent difference in the ΔF value of the tissue sample in the presence of the test compound and the averaged ΔF value of the vehicle control signal divided by the averaged ΔF value of the vehicle control signal.

Example 11

Smn Protein Assay in Tissues of Adult C/C-Allele SMA Mice

The tissues for use in the assay for Smn protein in adult C/C-allele SMA mice are prepared as described in Example 10. The assay assesses whether treatment of C/C-allele SMA mice with a test compound for 10 days increases levels of Smn protein produced from the SMN2 gene.

Materials

Material	Source
Tissues from C/C-allele SMA mice	The Jackson Laboratory, strain No.: 008714 (B6.129-Smn1 ^{tm5(Smn1)/SMN2^{Mrph}/J})

Protocol.

C/C-allele SMA mice are dosed BID orally (in 0.5% hydroxypropylmethyl cellulose (HPMC) with 0.1% Tween-80) with a test compound or vehicle at 10 mg/kg for 10 days. Age-matched heterozygous mice are dosed with vehicle for use as a control. Tissues are collected for analysis of protein levels according to Example 10.

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Results.

As seen in FIG. 11, total protein normalized Smn level was increased in brain, spinal cord, muscle and liver tissues of adult C/C-allele SMA mice treated at 10 mg/kg BID for 10 days with Compound 65 (FIG. 11a) and Compound 69 (FIG. 11b) relative to the vehicle group.

Example 12

Smn Protein in Tissues of Neonatal Δ7 SMA Mice

The assay for Smn protein in neonatal SMA mice tissues is used to determine whether treatment with a test compound increases Smn protein levels produced from the SMN2 gene.

Materials

Material	Source
Tissues from Δ7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1(Msd)/J})

Protocol.

SMA Δ7 homozygous knockout mice are dosed once a day (QD) intraperitoneally (IP) with a test compound or vehicle (100% DMSO) from postnatal day (PND) 3 to PND 9. Tissues are collected for analysis of protein levels according to Example 10.

Results.

As seen in FIG. 12, total protein normalized Smn level was dose dependently increased in brain (FIG. 12a), spinal cord (FIG. 12b) and muscle (FIG. 12c) tissues of neonatal SMA Δ7 homozygous knockout mice treated for 7 days QD with the indicated doses of Compound 65.

Example 13

Body Weight of Neonatal Δ7 SMA Mice

The change in body weight of neonatal SMA mice is used to determine whether treatment with a test compound improves body weight.

Materials

Material	Source
Tissues from ΔExon7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1(Msd)/J})

Protocol.

SMA Δ7 homozygous knockout mice are dosed intraperitoneally (IP) with test compound or vehicle (100% DMSO) QD from PND 3 until the dose regimen is switched to an oral dose BID in 0.5% hydroxypropylmethyl cellulose (HPMC) with 0.1% Tween-80 at a dose 3.16-fold higher than the dose used for IP. Body weights of SMA Δ7 mice treated with test compound or vehicle and age matched heterozygous mice are recorded every day.

Results.

As seen in FIG. 13, body weight of neonatal SMA Δ7 homozygous knockout mice treated with Compound 65 (FIG. 13a) and Compound 69 (FIG. 13b), dosed IP QD from PND 3 to PND 23, then orally BID from PND 24 until study end, improved compared to vehicle treated mice.

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Example 14

Righting Reflex in Neonatal $\Delta 7$ SMA Mice

The functional change in righting reflex of neonatal SMA mice is used to determine whether treatment with a test compound improves righting reflex.

Materials

Material	Source
Tissues from Δ Exon7 SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1Msd/J})

Protocol.

SMA $\Delta 7$ homozygous knockout mice are dosed intraperitoneally (IP) with test compound or vehicle (100% DMSO) QD from PND 3 until the dose regimen is switched to an oral dose BID in 0.5% hydroxypropylmethyl cellulose (HPMC) with 0.1% Tween-80 at a dose 3.16-fold higher than the dose used for IP. The righting reflex time is measured as the time taken by a mouse to flip over onto its feet after being laid on its back. Righting reflex is measured five times for each mouse (allowing a maximal time of 30 sec for each try) with 5 minutes between each measurement. The righting reflex time for SMA $\Delta 7$ homozygous knockout mice treated with test compound or vehicle and age-matched heterozygous mice is measured on PND 10, 14 and 18 and plotted.

Results.

As seen in FIG. 14, the righting reflex of neonatal SMA $\Delta 7$ homozygous knockout mice treated with Compound 65 (FIG. 15a) and Compound 69 (FIG. 15b) dosed IP QD from PND 3 improved compared to vehicle treated mice. The righting time of the compound treated neonatal SMA $\Delta 7$ homozygous knockout mice was similar to that of the age matched heterozygous mice on PND 18.

Example 15

Survival of Neonatal $\Delta 7$ SMA Mice

The change in the number of surviving mice over time is used to determine whether treatment with a test compound improves survival.

Materials

Material	Source
Tissues from $\Delta 7$ SMA mice	The Jackson Laboratory, strain No.: 005025 (FVB.Cg-Tg(SMN2*delta7)4299Ahmb Tg(SMN2)89Ahmb Smn1 ^{tm1Msd/J})

Protocol.

SMA $\Delta 7$ homozygous knockout mice are dosed intraperitoneally (IP) with test compound or vehicle (100% DMSO) QD from PND 3 until the dose regimen is switched to an oral dose BID in 0.5% hydroxypropylmethyl cellulose (HPMC) with 0.1% Tween-80 at a dose 3.16-fold higher than the dose used for IP and later switched to an oral dose QD in 0.5% hydroxypropylmethyl cellulose (HPMC) with 0.1% Tween-80 at a dose 6.32-fold higher than the dose used for IP. The number of surviving mice in each group is recorded every day and plotted as a percent of total number of mice. Tissues of SMA $\Delta 7$ and age-matched heterozygous mice are collected for the measurement of Smn protein levels and processed as

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detailed in Example 10. The total protein normalized Smn protein levels measured in the tissues are plotted as a percent of those in the age-matched heterozygous mice tissues, with the Smn level in heterozygous mice set to 100 percent. The level of Smn protein in the test compound treated mice tissue relative to that in heterozygous mice tissue is indicated as a percent value above each bar in the graph.

Results.

As seen in FIG. 15, survival of neonatal SMA $\Delta 7$ homozygous knockout mice treated with Compound 65 (FIG. 15a) and Compound 69 (FIG. 15b), dosed IP QD from PND 3 to PND 23, then orally BID from PND 24 until study end, improved compared to vehicle treated mice. As seen in FIG. 16, Smn protein levels in brain and muscle tissues of SMA $\Delta 7$ homozygous knockout mice after treatment with Compound 65 (FIG. 16a) until PND 144 and Compound 69 (FIG. 16b) from PND 3 until PND 80 and 83 was measured and plotted relative to vehicle treated and age-matched heterozygous mice.

Example 16

Human SMN1 Minigene mRNA End-Point Semi-Quantitative RT-PCR Splicing Assay in Cultured Cells

The RT-PCR assay is used to visualize and quantify the levels of the human SMN1 minigene full length and $\Delta 7$ mRNA in primary cells and cell lines expressing the human SMN1 minigene construct treated with a test compound.

Materials

Material	Source
HEK293H cells	ATCC Catalog No.: CRL-1573
Cells-To-Ct lysis buffer	Life Technologies, Inc. (formerly Applied Biosystems) Catalog No.: 4399002
FuGENE-6 lipid transfection reagent	Roche Applied Science, Catalog No.: 11 814 443 001
DMEM	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11960-044
96-well flat-bottom plates	Becton Dickinson Catalog No.: 353072
Platinum Taq HiFi DNA Polymerase Super Mix	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: 11304-016
iScript RT enzyme kit	BioRad Catalog No.: 170-8890
Ethidium bromide 2% agarose E gels 48-Well Double Comb	Life Technologies, Inc. (formerly Invitrogen) Catalog No.: G8008-02
Gel Documentation System	UVP Gel Doc It 310 Imaging system

SMN1 Minigene Construct

Preparation of the Minigene Constructs

Using the procedure for the preparation of the SMN2 minigene construct described in Biological Example 1, the SMN1 version of the minigene is generated by changing the sixth nucleotide of exon 7 (a thymine residue) of the SMN2-A minigene construct to cytosine using site directed mutagenesis. Thus, similar to the SMN2-A minigene construct, the SMN1 minigene construct has a single adenine residue inserted after nucleic residue 48 of exon 7. The SMN1 minigene construct is referred to as SMN1-A.

Protocol.

HEK293H cells (10,000 cells/well/199 μ L) were transfected, using FuGENE-6 reagent, in a 96-well plate with 15 ng of the SMN1-A minigene reporter plasmid per well. Cells were incubated for 24 hours following transfection. Test compounds were serially diluted 3.16-fold in 100% DMSO to generate a 7-point concentration curve. A solution of test

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compound (1 μ L, 200 \times in DMSO) was added to each test well. 1 μ L DMSO was added to each control well. The plate was incubated for 7 hours in a cell culture incubator (37° C., 5% CO₂, 100% relative humidity). The cells were then lysed in Cells-To-Ct lysis buffer and the lysates were stored at 80° C.

Two SMN spliced mRNA are generated from the SMN1 minigene. The term "SMN1 minigene FL" refers to the first spliced product containing exon 7, corresponding to full length SMN1 mRNA. The term "SMN1 minigene Δ 7" refers to the second product lacking exon 7.

SMN minigene FL and Δ 7 mRNA are amplified using the primers in Table 11. Primer SMN Forward C (SEQ ID NO. 11) hybridizes to a nucleotide sequence in exon 6 (nucleotide 43 to nucleotide 63), primer SMN Reverse A (SEQ ID NO. 2) hybridizes to a nucleotide sequence in the coding sequence of Firefly luciferase. The combination of these two oligonucleotides detects only SMN1 or SMN2 minigenes (RT-PCR) and will not detect endogenous SMN1 or SMN2 genes. Since the SMA patient cells used in Example 16 were transfected with only the human SMN1 minigene, RT-PCR can visualize and quantify only SMN1 minigene full-length and SMN1 minigene Δ 7 mRNA.

TABLE 11

Primer	Sequence	Source
SMN Forward C	SEQ ID NO. 11: GATGCTGATGCTTTGGGAAGT	PTC ¹
SMN Reverse A	SEQ ID NO. 2: CGCTTCACATTCCAGATCTGTC	PTC ¹

¹Primers designed by PTC Therapeutics, Inc.

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To synthesize cDNA, 5 μ L of lysate, 4 μ L of 5 \times iScript reaction mix, 1 μ L of reverse transcriptase, and 10 μ L of water are combined and incubated 5 min at 25° C. followed by 30 min at 42° C., followed by 5 min at 85° C. The cDNA solution is stored at -20° C.

To perform endpoint PCR, 5 μ L of cDNA, 0.2 μ L of 100 μ M forward primer, 0.2 μ L of 100 μ M reverse primer, and 22.5 μ L of polymerase super mix are combined in a 96 well semiskirted PCR plate. PCR is carried out at the following temperatures for the indicated time: Step 1: 94° C. (2 min), Step 2: 94° C. (30 sec), Step 3: 55° C. (30 sec), Step 4: 68° C. (1 min), then repeat Steps 2 to 4 for a total of 33 cycles, then hold at 4° C.

10 μ L of each PCR sample is electrophoretically separated on a 2% agarose E-gel for 14 minutes stained with dsDNA staining reagents (e.g., ethidium bromide) and visualized using a gel imager.

Results.

As seen in FIG. 17, cells treated with increasing concentrations of Compound 65 (FIG. 17a) and Compound 69 (FIG. 17b) contain progressively more SMN1 minigene FL mRNA and less SMN1 minigene Δ 7 mRNA, indicating a correction of SMN1 alternative splicing.

Without regard to whether a document cited herein is specifically and individually indicated as being incorporated by reference, all documents referred to herein are incorporated by reference into the present application for any and all purposes to the same extent as if each individual reference was fully set forth herein.

Although certain embodiments have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims as described herein.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 21

<210> SEQ ID NO 1
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
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19

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22

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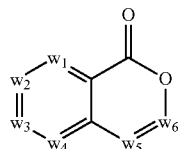
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What is claimed is:

1. A compound of Formula (I):



or a form thereof, wherein:

w₁ is C—R_b;w₂ and w₆ are C—R₁ or C—R₂;w₃, w₄ and w₅ are C—R_a;

wherein one of w₂ and w₆ is C—R₁ and the other is C—R₂,
 provided that, when w₂ is C—R₁, then w₆ is C—R₂; or,
 when w₂ is C—R₂, then w₆ is C—R₁;

R₁ is C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-
 amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈
 alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)₂-
 amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl,
 (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, C₁₋₈alkoxy-C₁₋₈alkyl-
 amino-C₁₋₈alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino-C₁₋₈
 alkyl, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈
 alkyl, amino-C₁₋₈alkyl-amino, (amino-C₁₋₈alkyl)₂-
 amino, (amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-
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 alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy,
 C₁₋₈alkoxy-C₁₋₈alkyl-amino-C₁₋₈alkoxy, (C₁₋₈alkoxy-
 C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy, amino-C₂₋₈
 alkenyl, C₁₋₈alkyl-amino-C₂₋₈alkenyl, (C₁₋₈alkyl)₂-
 amino-C₂₋₈alkenyl, amino-C₂₋₈alkynyl, C₁₋₈alkyl-
 amino-C₂₋₈alkynyl, (C₁₋₈alkyl)₂-amino-C₂₋₈alkynyl,
 halo-C₁₋₈alkyl-amino, (halo-C₁₋₈alkyl)₂-amino, (halo-
 C₁₋₈alkyl)(C₁₋₈alkyl)amino, hydroxy-C₁₋₈alkoxy-C₁₋₈
 alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈
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hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈
 alkyl)₂-amino-C₁₋₈alkyl, (hydroxy-C₁₋₈alkyl)(C₁₋₈
 alkyl)amino-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino-C₁₋₈
 alkoxy, (hydroxy-C₁₋₈alkyl)₂-amino-C₁₋₈alkoxy, (hy-
 droxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkoxy,
 hydroxy-C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (hydroxy-
 C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (hydroxy-C₁₋₈
 alkyl)₂-amino-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl-
 amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, (hydroxy-C₁₋₈
 alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl-amino, [(hydroxy-
 C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, [(hy-
 droxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl](C₁₋₈
 alkyl)amino, heterocyclyl, heterocyclyl-C₁₋₈alkyl, het-
 erocyclyl-C₁₋₈alkoxy, heterocyclyl-amino, (heterocy-
 clyl)(C₁₋₈alkyl)amino, heterocyclyl-amino-C₁₋₈alkyl,
 heterocyclyl-C₁₋₈alkyl-amino, (heterocyclyl-
 C₁₋₈alkyl)₂-amino, (heterocyclyl-C₁₋₈alkyl)(C₁₋₈alkyl)
 amino, heterocyclyl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (het-
 erocyclyl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (heterocyclyl-
 C₁₋₈alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heterocyclyl-
 oxy, heterocyclyl-carbonyl, heterocyclyl-carbonyl-oxy,
 aryl-C₁₋₈alkyl-amino, (aryl-C₁₋₈alkyl)₂-amino, (aryl-
 C₁₋₈alkyl)(C₁₋₈alkyl)amino, aryl-C₁₋₈alkyl-amino-C₁₋₈
 alkyl, (aryl-C₁₋₈alkyl)₂-amino-C₁₋₈alkyl, (aryl-C₁₋₈
 alkyl)(C₁₋₈alkyl)amino-C₁₋₈alkyl, heteroaryl, het-
 eroaryl-C₁₋₈alkyl, heteroaryl-C₁₋₈alkoxy, heteroaryl-
 amino, heteroaryl-C₁₋₈alkyl-amino, (heteroaryl-C₁₋₈
 alkyl)₂-amino, (heteroaryl-C₁₋₈alkyl)(C₁₋₈alkyl)amino,
 heteroaryl-C₁₋₈alkyl-amino-C₁₋₈alkyl, (heteroaryl-C₁₋₈
 alkyl)₂-amino-C₁₋₈alkyl or (heteroaryl-C₁₋₈alkyl)(C₁₋₈
 alkyl)amino-C₁₋₈alkyl;

wherein, each instance of heterocyclyl and heteroaryl is
 optionally substituted with one, two or three R₃ substitu-
 ents and one additional, optional R₄ substituent; and,
 wherein, alternatively, each instance of heterocyclyl and
 heteroaryl is optionally substituted with one, two, three
 or four R₃ substituents;

R₂ is aryl, aryl-amino, aryl-amino-carbonyl, heterocyclyl,
 heteroaryl or heteroaryl-amino;

wherein, each instance of aryl, heterocyclyl and heteroaryl
 is optionally substituted with one, two or three R₆ sub-
 stituents and one additional, optional R₇ substituent;

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R_a is, in each instance, independently selected from hydrogen, halogen or C₁₋₈alkyl;

R_b is hydrogen, halogen, C₁₋₈alkyl or C₁₋₈alkoxy;

R₃ is, in each instance, independently selected from cyano, halogen, hydroxy, oxo, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkyl-carbonyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, C₁₋₈alkoxy-carbonyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino, amino-C₁₋₈alkyl, C₁₋₈alkyl-amino-C₁₋₈alkyl, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, C₁₋₈alkyl-amino-C₁₋₈alkyl-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)₂-amino, (C₁₋₈alkyl)₂-amino-C₁₋₈alkyl-amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl]₂-amino, (C₁₋₈alkyl-amino-C₁₋₈alkyl)(C₁₋₈alkyl)amino, [(C₁₋₈alkyl)₂-amino-C₁₋₈alkyl](C₁₋₈alkyl)amino, C₁₋₈alkoxy-C₁₋₈alkyl-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)₂-amino, (C₁₋₈alkoxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino, C₁₋₈alkyl-carbonyl-amino, C₁₋₈alkoxy-carbonyl-amino, hydroxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkoxy-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl-amino, (hydroxy-C₁₋₈alkyl)₂-amino or (hydroxy-C₁₋₈alkyl)(C₁₋₈alkyl)amino;

R₄ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-C₁₋₈alkyl, C₃₋₁₄cycloalkyl-amino, aryl-C₁₋₈alkyl, aryl-C₁₋₈alkoxy-carbonyl, aryl-sulfonyloxy-C₁₋₈alkyl, heterocyclyl or heterocyclyl-C₁₋₈alkyl; wherein, each instance of C₃₋₁₄cycloalkyl, aryl and heterocyclyl is optionally substituted with one, two or three R₅ substituents;

R₅ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, halo-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio;

R₆ is, in each instance, independently selected from halogen, hydroxy, cyano, nitro, C₁₋₈alkyl, C₂₋₈alkenyl, halo-C₁₋₈alkyl, hydroxy-C₁₋₈alkyl, C₁₋₈alkoxy, halo-C₁₋₈alkoxy, C₁₋₈alkoxy-C₁₋₈alkyl, amino, C₁₋₈alkyl-amino, (C₁₋₈alkyl)₂-amino or C₁₋₈alkyl-thio; and,

R₇ is C₃₋₁₄cycloalkyl, C₃₋₁₄cycloalkyl-oxy, aryl, heterocyclyl or heteroaryl.

2. The compound of claim 1, wherein the form is selected from a free acid, free base, salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

3. The compound of claim 1, wherein the salt form is a chloride, hydrochloride, dihydrochloride, hydrobromide, acetate or trifluoroacetate salt.

4. The compound of claim 1, wherein the compound is selected from:

- 7-(piperazin-1-yl)-3-(pyridin-2-yl)-1H-isochromen-1-one;
- 7-(piperazin-1-yl)-3-(thiophen-3-yl)-1H-isochromen-1-one;
- 3-(3,4-dimethoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 7-(4-methylpiperazin-1-yl)-3-(pyridin-2-yl)-1H-isochromen-1-one;
- 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(pyridin-2-yl)-1H-isochromen-1-one;
- 3-(2,2-difluoro-1,3-benzodioxol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(2,2-difluoro-1,3-benzodioxol-5-yl)-7-(4-methyl-1,4-diazepan-1-yl)-1H-isochromen-1-one;
- 3-(1,3-benzothiazol-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(1,3-benzothiazol-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(1,3-benzothiazol-2-yl)-7-(1,4-diazepan-1-yl)-1H-isochromen-1-one;

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- 3-(1,3-benzothiazol-2-yl)-7-(4-methyl-1,4-diazepan-1-yl)-1H-isochromen-1-one;
- 3-(1,3-benzodioxol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(3,5-difluorophenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(1,3-benzodioxol-5-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 3-(3,4-dimethoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 3-(3,4-dimethoxyphenyl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(3-methoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(3-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(3-methoxyphenyl)-1H-isochromen-1-one;
- 7-(1,4-diazepan-1-yl)-3-(3-methoxyphenyl)-1H-isochromen-1-one;
- 3-(2-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(2-methoxyphenyl)-1H-isochromen-1-one;
- 3-(4-methoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(4-methoxyphenyl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 3-(imidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 3-(imidazo[1,2-a]pyridin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
- 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(4-methoxyphenyl)-1H-isochromen-1-one;
- 3-(3,4-dimethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(2-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 7-[(3S)-3-methylpiperazin-1-yl]-3-(pyridin-2-yl)-1H-isochromen-1-one;
- 3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(1,3-benzothiazol-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
- 3-(2,3-dihydro-1,4-benzodioxin-6-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(1,3-benzodioxol-5-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(3,4-dihydroxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(4-ethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
- 3-(3-fluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;

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3-(3-hydroxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(2-fluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(3-chloro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(4-fluoro-3-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(5-fluoro-2-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(3,5-difluoro-4-methoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(2,4-dimethoxyphenyl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(4-ethoxyphenyl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-1-benzofuran-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-[3-(difluoromethoxy)phenyl]-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-[4-(difluoromethoxy)phenyl]-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(imidazo[2,1-b][1,3]thiazol-6-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(8-chloroimidazo[1,2-a]pyridin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(3,4-dimethoxyphenyl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-hydroxyethyl)piperazin-1-yl]-1H-isochromen-1-one;

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3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[4-(2-hydroxyethyl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[(8aS)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one;
 3-(1,3-dimethylpyrrolo[1,2-a]pyrazin-7-yl)-7-[(8aR)-hexahydropyrrolo[1,2-a]pyrazin-2(1H)-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-propylpiperazin-1-yl)-1H-isochromen-1-one;
 7-(4-tert-butylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-(4-cyclopropylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-methoxyethyl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(2-methylpropyl)piperazin-1-yl]-1H-isochromen-1-one;
 7-(4-cyclobutylpiperazin-1-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-ethyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(propan-2-yl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(2-methoxyethyl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one;
 7-(1-cyclopropyl-1,2,3,6-tetrahydropyridin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-(1-cyclobutyl-1,2,3,6-tetrahydropyridin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-propyl-1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(piperidin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(oxetan-3-yl)-1,2,3,6-tetrahydropyridin-4-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-ethylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-1,3-benzothiazol-6-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-1,3-benzothiazol-6-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-1,3-benzothiazol-6-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;

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3-(2-methyl-1,3-benzothiazol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-1,3-benzothiazol-6-yl)-7-[4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3,4-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6-chloro-8-methylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-methylpiperidin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-ethylpiperidin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(1-propylpiperidin-4-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(propan-2-yl)piperidin-4-yl]-1H-isochromen-1-one;
 7-(1-cyclobutylpiperidin-4-yl)-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[1-(oxetan-3-yl)piperidin-4-yl]-1H-isochromen-1-one;
 7-[(3S)-4-ethyl-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-[(3S)-3,4-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-(4-hydroxypiperidin-1-yl)-1H-isochromen-1-one;
 7-[4-(dimethylamino)piperidin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R)-4-ethyl-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-[(3R)-3,4-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R,5S)-4-ethyl-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-[(3R,5S)-4-(2-hydroxyethyl)-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R,5S)-4-cyclobutyl-3,5-dimethylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-[(3R)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-3-(6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;

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3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-ethyl-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3S)-4-cyclobutyl-3-methylpiperazin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methyl-4-(oxetan-3-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-ethyl-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-(2-hydroxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R)-4-cyclobutyl-3-methylpiperazin-1-yl]-3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methyl-4-(propan-2-yl)piperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-4-(2-methoxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-(2-methoxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-4-(2-methoxyethyl)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-4-ethyl-3,5-dimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,5-dimethyl-4-propylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(6,8-dimethylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3S)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R)-3-methylpiperazin-1-yl]-1H-isochromen-1-one;
 7-[(3R,5S)-3,5-dimethylpiperazin-1-yl]-3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 7-[(3S)-3,4-dimethylpiperazin-1-yl]-3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-1H-isochromen-1-one;
 3-(2-methyl-2H-indazol-5-yl)-7-(piperazin-1-yl)-1H-isochromen-1-one;
 3-(2-methyl-2H-indazol-5-yl)-7-(4-methylpiperazin-1-yl)-1H-isochromen-1-one;
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-[(3R,5S)-3,4,5-trimethylpiperazin-1-yl]-1H-isochromen-1-one; or
 3-(8-ethyl-6-methylimidazo[1,2-a]pyrazin-2-yl)-7-(1,2,3,6-tetrahydropyridin-4-yl)-1H-isochromen-1-one, or a salt, isotopologue, stereoisomer, racemate, enantiomer, diastereomer or tautomer thereof.

5. A pharmaceutical composition comprising an effective amount of the compound of claim 1 and a pharmaceutically acceptable carrier, excipient or diluent.

6. The pharmaceutical composition of claim 5 for use in the treatment of spinal muscular atrophy (SMA).

7. A method for enhancing the inclusion of exon 7 of SMN2 into mRNA that is transcribed from the SMN2 gene, comprising contacting a human cell with the compound of claim 1. 5

8. A method for increasing the amount of Smn protein, comprising contacting a human cell with the compound of claim 1.

9. The method of claim 7 or 8, wherein the human cell is a human cell from a human SMA patient. 10

10. A method for treating SMA in a human subject in need thereof, comprising administering to the subject an effective amount of the compound of claim 1.

11. A method for treating SMA in a human subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 5. 15

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